PB86-102597

Health Assessment Document for Ethylene Oxide
Final Report

(U.S.) Environmental Protection Agency
Washington, DC

Jun 85

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA/600/8-84/009F

2.

3. RECIPIENT'S ACCESSION NO.
PB86 102597

4. TITLE AND SUBTITLE
Health Assessment Document for Ethylene Oxide:
Final Report

5. REPORT DATE
June 1985

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
See list of authors and contributors

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Health and Environmental Assessment (OHEA)
Environmental Criteria and Assessment Office (MD-52)
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED
Final Report

14. SPONSORING AGENCY CODE
EPA/600/23

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The largest single use of ethylene oxide is as an intermediate in the synthesis of ethylene glycol. However, small amounts of this epoxide are used as a sterilant or pesticide in commodities, pharmaceuticals, medical devices, tobacco, and other items, representing a considerable potential for human exposure.

The pharmacokinetics of ethylene oxide have not been studied extensively. While only limited studies found in the literature searched concerned the absorption of this chemical, toxicity data suggest that absorption occurs via the respiratory and gastrointestinal tracts.

Acute exposure to ethylene oxide in humans results in symptoms of headache, vomiting, dyspnea, and diarrhea. Subacute and chronic effects are not well documented.

Data indicate that many different types of genetic damage may be induced following exposure to ethylene oxide. It was found to be mutagenic in the Ames Salmonella assay to Bacillus subtilis and to Drosophila melanogaster, as well as in cultured mammalian cell and assay systems.

The alkylating and mutagenic properties of ethylene oxide are sufficient causes of concern about its potential carcinogenicity. Though there are no definitive, epidemiologic studies, the data are consistent with the animal data in that there seems to be a strong association between an increased risk of cancer, especially leukemia, and prolonged inhalation exposure to ethylene oxide.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
Release to Public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
308

22. PRICE

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA/600/8-84/009F
June 1985
Final Report

Research and Development

EPA

Health Assessment Document for Ethylene Oxide

Final Report

PB86-102597

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/8-84-009F

Health Assessment Document for Ethylene Oxide

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

DISCLAIMER

This document has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. The health assessment document was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of ethylene oxide as a hazardous air pollutant. However, the scope of this document has since been expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

AUTHORS, CONTRIBUTORS AND REVIEWERS

AUTHORS:

The EPA's Office of Health and Environmental Assessment (OHEA) is responsible for the preparation of the health assessment document. The OHEA Environmental Criteria and Assessment Office (ECAO-RTP) had overall responsibility for coordination and direction of the document (Dr. Robert M. Bruce, Project Manager). The chapters addressing physical and chemical properties, sampling and analysis, air quality, and biological effects in animals and man were originally written and revised by Syracuse Research Corporation with the exception of chapters or sections addressing mutagenicity, teratogenicity and reproductive effects and carcinogenicity. The air quality chapters (5, 6, 7) were reviewed by Radian Corporation under contract to the Office of Air Quality Planning and Standards and recommendations proposed.

The principal authors of the chapters or sections prepared by Syracuse Research Corporation are:

D. Anthony Gray, Ph.D.
Life and Environmental Sciences Division
Syracuse Research Corporation
Syracuse, NY

Bruce Harris, Ph.D
Life and Environmental Sciences Division
Syracuse Research Corporation
Syracuse, NY

Stephen Bosch
Life and Environmental Sciences Division
Syracuse Research Corporation
Syracuse, NY

Joseph Santodonato, Ph.D, CIH
Life and Environmental Sciences Division
Syracuse Research Corporation
Syracuse, NY

The OHEA Carcinogen Assessment Group (CAG) was responsible for preparation of the sections on carcinogenicity. Participating members of the CAG are listed below (principal authors of present carcinogenicity materials are designated by an asterisk(*).

Roy E. Albert, M.D. (Chairman)
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.*
David L. Bayliss, M.S.
Robert P. Beliles, Ph.D.
Chao W. Chen, Ph.D.
Margaret M.L. Chu, Ph.D.
Herman J. Gibb, B.S., M.P.H.*
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Jean C. Parker, Ph.D
Dharm V. Singh, D.V.M., Ph.D.*
Todd W. Thorslund, Sc.D.

The OHEA Reproductive Effects Assessment Group (REAG) was responsible for the preparation of sections on mutagenicity, teratogenicity and reproductive effects. Participating members of REAG are listed below (principal authors of present sections are indicated by an asterisk). The Environmental Mutagen Information Center (EMIC), in Oak Ridge, TN, identified literature bearing on the mutagenicity of ethylene oxide.

Eric Clegg, Ph.D.*
John R. Fowle, III, Ph.D.*
Ernest R. Jackson, M.S.
Casey Jason, M.D.
David Jacobson-Kram, Ph.D.
K.S. Lavappa, Ph.D.
Sheila L. Rosenthal, Ph.D.

Carol N. Sakai, Ph.D.*
Carmella Tellone, B.S.*
Vicki-Vaughan Dellarco, Ph.D.
Peter E. Voytek, Ph.D (Director)

REVIEWERS

The following individuals provided peer review of this draft or earlier drafts of this document:

U.S. Environmental Protection Agency

Karen Blanchard
Office of Air, Noise and Radiation
Office of Air Quality Planning and Standards
Research Triangle Park, NC

Robert M. Bruce, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC

James W. Falco, Ph.D.
Office of Health and Environmental Assessment
Exposure Assessment Group
Washington, D.C.

Lester D. Grant, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC

Joseph Padgett
Office of Air, Noise and Radiation
Office of Air Quality Planning and Standards
Research Triangle Park, NC

William E. Pepelko
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, OH

Jerry F. Stara, D.V.M.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, OH

Consultants and Reviewers

I.W.F. Davidson, Ph.D.
Bowman Gray School of Medicine
Wake Forest University
Winston Salem, NC

Larry Fishbein, Ph.D.
National Center for Toxicological Research
Jefferson, AR

Richard N. Hill, M.D., Ph.D.
Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, DC

Derek Hodgson, Ph.D.
University of North Carolina
Chapel Hill, NC

George R. Hoffman, Ph.D.
Holy Cross College
Worcester, MA

Rudolph J. Jaeger, Ph.D.
Consultant Toxicologist
7 Bogert Place
Westwood, NJ

Marshall Johnson, Ph.D.
Thomas Jefferson Medical College
Philadelphia, PA

Edmond J. LaVoie, Ph.D.
American Health Foundation
Valhalla, NY

P.D. Lotilaker, Ph.D.
Fels Research Institute
Temple University Medical Center
Philadelphia, PA

Sam Shibko, Ph.D.
Health and Human Services
Division of Toxicology
Washington, DC

Charles M. Sparacino, Ph.D.
Research Triangle Institute
Research Triangle Park, NC

Danial S. Straus, Ph.D.
University of California
Riverside, CA

Robert Tardiff, Ph.D.
1423 Trapline Court
Vienna, VA

Norman M. Trieff, Ph.D.
University of Texas Medical Branch
Department of Pathology, UTMB
Galveston, TX

Benjamin Van Duuren, Ph.D.
New York University Medical Center
550 First Avenue
New York, NY

James R. Withey, Ph.D.
Department of National Health and Welfare
Tunney's Pasture
Ottawa, Ontario
Canada, KIA 01Z

SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE

The content of this health assessment document on ethylene oxide was independently peer-reviewed in public session by the Environmental Health Committee of the Environmental Protection Agency's Science Advisory Board.

CHAIRMAN, ENVIRONMENTAL HEALTH COMMITTEE

Dr. Herschel E. Griffin, Professor of Epidemiology, Graduate School of Public Health, 6505 Alvarado Road, San Diego State University, San Diego, California 92182-0405

EXECUTIVE SECRETARY, SCIENCE ADVISORY BOARD

Dr. Daniel Byrd III, Executive Secretary, Science Advisory Board, A-101 F, U.S. Environmental Protection Agency, Washington, D.C. 20460

MEMBERS

Dr. Seymour Abrahamson, Professor of Zoology and Genetics, Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

Dr. Morton Corn, Professor and Director, Division of Environmental Health Engineering, School of Hygiene and Public Health, The Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Maryland 21205

Dr. John Doull, Professor of Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, Kansas 66103

Dr. Jack D. Hackney, Chief, Environmental Health Laboratories, Professor of Medicine, Rancho Los Amigos Hospital Campus of the University of Southern California, 7601 Imperial Highway, Downey, California 90242

Dr. Marvin Kuschner, Dean, School of Medicine, Health Science Center, Level 4, State University of New York, Stony Brook, New York 11794

Dr. Daniel Menzel, Director and Professor, Pharmacology and Medicine, Director, Cancer Toxicology and Chemical Carcinogenesis Program, Duke University Medical Center, Durham, North Carolina 27710.

Dr. Steven M. Rappaport, Associate Professor of Industrial Hygiene, School of Public Health, Department of Biomedical and Environmental Health Sciences, University of California, Berkeley

Dr. Michael J. Symons, Professor, Department of Biostatistics, School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27711

Dr. D. Warner North, Principal, Decision Focus Inc., Los Altos Office Center, Suite 200, 4984 El Camino Real, Los Altos, California 94022

Dr. Bernard Weiss, Professor, Division of Toxicology, P.O. Box RBB, University of Rochester, School of Medicine, Rochester, New York 14642

Dr. Ronald Wyzga, Electric Power Research Institute, 3412 Hillview Avenue, P.O. Box 1041, Palo Alto, California 94303

Dr. Edward F. Ferrand, Assistant Commissioner for Science and Technology, New York City Department of Environmental Protection, 51 Astor Place, New York, New York 10003

Dr. Ronald D. Hood, Professor, Developmental Biology Section, Department of Biology, The University of Alabama, and Principal Associate, R.D. Hood and Associates, Consulting Toxicologists, P.O. Box 1927, University, Alabama 35486

SPECIAL CONSULTANTS

Dr. Robert R. Maronpot, Head, Experimental Pathology, National Toxicology Program, NIEHS, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Dr. Walderico M. Generoso, Senior Scientist, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

TABLE OF CONTENTS

Page

LIST OF TABLES.. xv

LIST OF FIGURES... xix

LIST OF ABBREVIATIONS... xx

1. SUMMARY AND CONCLUSIONS....................................... 1-1

2. INTRODUCTION.. 2-1

3. PHYSICAL AND CHEMICAL PROPERTIES.............................. 3-1

3.1 SYNONYMS AND CAS NUMBER...................................... 3-1
3.2 STRUCTURAL AND MOLECULAR FORMULAS............................ 3-1
3.3 TORSIONAL ANGLES AND BOND DISTANCES.......................... 3-1
3.4 PHYSICAL PROPERTIES OF PURE ETHYLENE OXIDE................... 3-2

3.4.1 Description.. 3-2
3.4.2 Molecular Weight... 3-2
3.4.3 Melting Point.. 3-2
3.4.4 Boiling Point.. 3-2
3.4.5 Boiling Point Change with Pressure Change.................. 3-2
3.4.6 Density.. 3-2
3.4.7 Coefficient of Cubical Expansion........................... 3-2
3.4.8 Refractive Index... 3-3
3.4.9 Vapor Pressure... 3-3
3.4.10 Aqueous Solubility.. 3-3
3.4.11 Freezing Point of Aqueous Solutions....................... 3-4
3.4.12 Boiling Point of Aqueous Solutions........................ 3-4
3.4.13 Flash Point... 3-4
3.4.14 Flash Point of Aqueous Solutions.......................... 3-5
3.4.15 Explosive Limits in Air, Volume %......................... 3-5
3.4.16 Heat of Combustion at 25°C................................ 3-5
3.4.17 Log Octanol/Water Partition Coefficient................... 3-5
3.4.18 Ultraviolet Spectroscopic Data............................ 3-5

3.5 PHYSICAL PROPERTIES AND DESCRIPTION OF COMMERCIAL ETHYLENE OXIDE.......... 3-5
3.6 CHEMICAL PROPERTIES.. 3-7

3.6.1 Reduction.. 3-7
3.6.2 Clathrate Formation.. 3-7

TABLE OF CONTENTS (cont.)

Page

3.6.3 Polymerization.. 3-7
3.6.4 Other Reactions.. 3-8
3.6.5 Hydrolysis and Related Reactions.. 3-8
3.6.6 Free Radical Reactions.. 3-17

4. SAMPLING AND ANALYTICAL METHODS.. 4-1

4.1 SAMPLING.. 4-1
4.2 ANALYSIS.. 4-6

5. SOURCES IN THE ENVIRONMENT.. 5-1

5.1 PRODUCTION.. 5-1

5.1.1 Quantities Produced.. 5-1
5.1.2 Producers, Production Sites, and Distribution...... 5-1
5.1.3 Production Methods and Processes.. 5-1

5.2 USES OF ETHYLENE OXIDE.. 5-13

5.2.1 Ethylene Glycol.. 5-13
5.2.2 Nonionic Surface-Active Agents.. 5-15
5.2.3 Di-, Tri-, and Polyethylene Glycols.. 5-15
5.2.4 Glycol Ethers.. 5-16
5.2.5 Ethanolamines.. 5-16
5.2.6 Miscellaneous Applications.. 5-16
5.2.7 Discontinued Uses of Epoxides.. 5-18
5.2.8 Projected or Proposed Uses.. 5-18
5.2.9 Alternatives to Uses for Ethylene Oxide.. 5-18

5.3 POTENTIAL FOR ENVIRONMENTAL CONTAMINATION.. 5-19

5.3.1 Air Emissions from Production.. 5-19
5.3.2 Handling, Transport, and Storage.. 5-22
5.3.3 Potential Environmental Formation.. 5-23

5.4 SUMMARY.. 5-25

6. ENVIRONMENTAL FATE, TRANSPORT, AND DISTRIBUTION.. 6-1

6.1 INTRODUCTION.. 6-1
6.2 ETHYLENE OXIDE FATE IN WATER.. 6-1
6.3 ETHYLENE OXIDE FATE IN SOIL.. 6-3

TABLE OF CONTENTS (cont.)

Page

6.4 ETHYLENE OXIDE FATE IN THE ATMOSPHERE 6-4
6.5 DEGRADATION IN COMMODITIES AND MANUFACTURED PRODUCTS 6-6
6.6 BIOACCUMULATION IN AQUATIC ORGANISMS 6-9
6.7 SUMMARY 6-9

7. ENVIRONMENTAL LEVELS AND EXPOSURE 7-1

7.1 INTRODUCTION 7-1
7.2 ENVIRONMENTAL LEVELS 7-1
7.3 EXPOSURE 7-4
7.4 SUMMARY 7-4

8. ECOLOGICAL EFFECTS 8-1

8.1 MICROORGANISMS AND INSECTS 8-1
8.2 PLANTS 8-2
8.3 AQUATIC ORGANISMS 8-2

9. BIOLOGICAL EFFECTS IN ANIMALS AND MAN 9-1

9.1 PHARMACOKINETICS 9-1

- 9.1.1 Absorption 9-1
- 9.1.2 Distribution 9-1
- 9.1.3 Metabolism 9-3
- 9.1.4 Elimination 9-4

9.2 ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY 9-6

- 9.2.1 Effects in Humans 9-6
- 9.2.2 Effects in Animals 9-14
- 9.2.3 Summary of Toxicity 9-24

9.3 TERATOGENICITY AND REPRODUCTIVE TOXICITY 9-26

- 9.3.1. Teratogenic Effects 9-26
- 9.3.2. Reproductive Effects 9-31
- 9.3.3. Testicular Effects 9-33
- 9.3.4. Adverse Reproductive Outcome in Humans 9-35
- 9.3.5. Summary of Teratogenicity and Reproductive Toxicity 9-38

TABLE OF CONTENTS (cont.)

Page

9.4 MUTAGENICITY.. 9-46

9.4.1. Gene Mutation Studies.................................... 9-49
9.4.2. Chromosome Aberration Studies............................ 9-64
9.4.3. Chromosome Mutations in Human Populations............ 9-77
9.4.4. Other Studies Indicative of Genetic Damage.......... 9-81
9.4.5. Summary and Conclusions of the Mutagenicity of Ethylene Oxide.. 9-92

9.5 CARCINOGENICITY.. 9-94

9.5.1 Animal Studies.. 9-94
9.5.2 Epidemiologic Studies....................................... 9-118
9.5.3 Quantitative Estimation..................................... 9-136
9.5.4 Summary.. 9-162
9.5.5 Conclusions.. 9-168

Appendix 9A: Comparison of Results by Various Extrapolation Models.. A1
Appendix 9B: International Agency for Research on Cancer Classification System for the Evaluation of the Carcinogenic Risk of Chemicals to Humans............ B1

10. REFERENCES.. 10-1

LIST OF TABLES

Table Page

3-1 Manufacturers' Specifications for Ethylene Oxide.............. 3-6

3-2 Typical Reactions of Ethylene Oxide.......................... 3-9

3-3 Hydrolysis Kinetics of Ethylene Oxide........................ 3-12

3-4 Specific Rates of Reaction of Anions and Lewis Bases with Ethylene Oxide.. 3-13

4-1 Breakthrough and Safe Sampling Volumes for Propylene Oxide with Several Sorbents.. 4-2

5-1 Ethylene Oxide Production.................................... 5-2

5-2 Ethylene Oxide Producers, Plant Sites, Capacities, Processes, and Technology.. 5-3

5-3 Ranges of Reaction Systems Variables in the Direct Air-Oxidation of Ethylene Oxide.............................. 5-9

5-4 Ranges of Reaction Systems Variables in the Direct Oxygen-Oxidation of Ethylene Oxide........................... 5-11

5-5 Users and Use Sites of Ethylene Oxide........................ 5-14

5-6 Typical Vent Gas Composition for Both Air- and Oxygen-Based Ethylene Oxide Plants...................................... 5-21

8-1 Acute Aquatic Toxicity of Ethylene Oxide..................... 8-3

9-1 Acute Toxicity of Ethylene Oxide............................. 9-2

9-2 Subchronic Toxicity of Ethylene Oxide........................ 9-17

9-3 Summary of Studies on Teratogenicity and Reproductive Toxicity... 9-39

9-4 Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutations in Bacteria....................................... 9-47

9-5 Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutations Tests in Lower Plants.(Yeast)...................... 9-53

9-6 Summary of Mutagenicity Testing of Ethylene Oxide: Mutation Tests in Higher Plants..................................... 9-55

LIST OF TABLES (cont.)

Table		Page
9-7	Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutation Tests in Insects	9-58
9-8	Summary of Mutagenicity Testing of Ethylene Oxide: Mammalian Cells in Culture	9-62
9-9	Summary of Mutagenicity Testing of Ethylene Oxide: Dominant Lethal Tests	9-65
9-10	Summary of Mutagenicity Testing of Ethylene Oxide: Heritable Translocation Test	9-67
9-11	Summary of Mutagenicity Testing of Ethylene Oxide: Chromosome Aberration Tests	9-68
9-12	Summary of Mutagenicity Testing of Ethylene Oxide: Micronucleus Tests	9-69
9-13	Summary of Mutagenicity Testing of Ethylene Oxide: Chromosome Mutations in Human Populations	9-70
9-14	Summary of Mutagenicity Testing of Ethylene Oxide: SCE Formation in Human Populations	9-82
9-15	Summary of Mutagenicity Testing of Ethylene Oxide: SCE Formation in Experimental Studies	9-84
9-16	Summary of Mutagenicity Testing of Ethylene Oxide: Unscheduled DNA Synthesis	9-85
9-17	Design Summary for Carcinogenicity Testing of Ethylene Oxide by Intragastric Administration to Sprague-Dawley Rats	9-97
9-18	Tumor Induction by Intragastric Administration of Ethylene Oxide in Female Sprague-Dawley Rats	9-97
9-19	Cumulative Percentages of Male Fischer 344 Rats that Died or were Sacrificed in a Moribund Condition After Exposure to Ethylene Oxide Vapor	9-101
9-20	Cumulative Percentages of Female Fischer 344 Rats that Died or were Sacrificed in a Moribund Condition After Exposure to Ethylene Oxide Vapor	9-102

LIST OF TABLES (cont.)

Table		Page
9-21	Cumulative Percentages of Male Fischer 344 Rats that were Alive at the Beginning of Month 17, but Died or were Sacrificed in a Moribund Condition After Subsequent Exposure to Ethylene Oxide Vapor	9-103
9-22	Cumulative Percentages of Female Fischer 344 Rats that were Alive at the Beginning of Month 17, but Died or were Sacrificed in a Moribund Condition After Subsequent Exposure to Ethylene Oxide Vapor	9-104
9-23	Summary of Selected Tumor Incidence Comparisons for Male and Female Fischer 344 Rats Exposed to Ethylene Oxide for Two Years	9-106
9-24	Ethylene Oxide 2-Year Vapor Inhalation Study: 24-Month Final Sacrificed Frequency of Exposure-Related Neoplasms for 110- to 116-Week-Old Fischer 344 Rats	9-107
9-25	Ethylene Oxide 2-Year Vapor Inhalation Study: Frequency of Exposure Related Neoplasms at 24-Month Final Sacrificed and in Fischer 344 Rats Dying Spontaneously or Euthanized When Moribund	9-108
9-26	Ethylene Oxide 2-Year Vapor Inhalation Study: Frequency of Brain Neoplasms in Fischer 344 Rats	9-112
9-27	Ethylene Oxide 2-Year Vapor Inhalation Study: Frequency of Primary Brain Neoplasms Types in Fischer 344 Rats	9-113
9-28	Leukemia Incidence in Male Fischer 344 Rats Exposed to Ethylene Oxide for 2 years	9-115
9-29	Incidence of Neoplastic Lesions in Male Fischer 344 Rats Exposed to Ethylene Oxide for 2 years	9-117
9-30	Observed and Expected Number of Deceased Among 153 Women and 50 Men with Continuous or Intermittent Exposure to Ethylene Oxide	9-127
9-31	Comparison of Observed Numbers of Cancer Deaths in BASF-Aktiengesellschaft, Ludwigshafen Plants 1928-80 for Persons Having 10 Years of Observation Following Exposure to Alkylene Oxide With That Expected Based on Mortality Statistics for Rhinehessia-Palatinate 1970-75, Ludwigshafen 1970-75, and the Federal Republic of Germany 1971-74, By ICD Code and Cause of Death	9-130

LIST OF TABLES (cont.)

Table		Page
9-32	Related Risks of Death From Cancer in the Alkylene Oxide Cohort as Compared With the Styrene Cohort, By Age............	9-132
9-33	Bushy Run Ethylene Oxide Inhalation Study in Fischer 344 Rats. Incidence of Peritoneal Mesothelioma and Brain Glioma in Males, and Mononuclear Cell Leukemia and Brain Glioma in Females by Dose Among Survivors to First Tumor. Maximum Liklihood Estimates of Linear Term and 95% Upper-Limit q_1^*...............	9-150
9-34	NIOSH Ethylene Oxide Inhalation Study in Male Fischer 344 Rats. Incidence of Peritoneal Mesothelioma and Brain Glioma by Dose Among Total Examined. Estimates of 95% Upper-Limit Risk Based on Human Equivalent Dose..................................	9-154
9-35	Leukemia (ICD 204-207) Incidence and Mortality: Ethylene Oxide Epidemiology. Included are Relative Risks, 95% Confidence Limits, Nominal Exposure Estimates, and 95% Confidence Limits on Unit Risk..	9-156
9-36	Relative Carcinogenic Potencies Among 54 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens..	9-163

LIST OF FIGURES

Figure Page

5-1 Schematic for Air-Based Ethylene Oxidation.................... 5-6

9-1 Mutagenic Response of Salmonella typhimurium Strain TA1535 Exposed to Ethylene Oxide........................... 9-50

9-2 Mutagenic Response of CHO Cells to EtO........................ 9-63

9-3 Percentages of Male and Female Fischer 344 Rats with Histologically Confirmed Mononuclear Cell Leukemia at 24-Month Sacrifice.................................... 9-111

9-4 Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group......................... 9-161

LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
BOD	Biochemical oxygen demand
CNS	Central nervous system
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
GC	Gas chromatography
GSH	Gluthathione
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
MS	Mass spectometry
ppb	Parts per billion
ppm	Parts per million
PVC	Polyvinyl chloride
SCE	Sister chromatid exchange
TWA	Time-weighted average
v/v	Volume per volume

1. SUMMARY AND CONCLUSIONS

Ethylene oxide is a colorless, flammable gas at ambient temperature. It is soluble in water (195 mℓ vapor in 1 mℓ water at 20°C) and is a highly reactive chemical. The chemical reaction of ethylene oxide that is environmentally significant is the hydrolytic reaction in aqueous media.

The most suitable chemical method available for the analysis of ethylene oxide in the atmosphere is its collection on a sorbent cartridge and subsequent analysis by gas chromatography with flame ionization or mass spectrometric detector. Biological samples are analyzed by the purge and trap method, followed by gas chromatography with flame ionization or mass spectrometric detection.

Ethylene oxide is produced almost exclusively by direct oxidation of ethylene. Its 1981 production volume was 4937 million pounds. The largest single use of ethylene oxide is in the synthesis of ethylene glycol. Small amounts of ethylene oxide are used as a sterilant and in the manufacture of pesticides, pharmaceuticals, and medicinal devices.

The major emission sources from production facilities of ethylene oxide are the main process vents and purge gas vents. Total air emission from production in 1978 has been estimated to be 2 million pounds. Although only small amounts of ethylene oxide are used for consumer products, this represents a considerable potential for human exposure.

In aquatic media, ethylene oxide will degrade by hydrolysis with a half-life of ≃ 12-14 days. Evaporation from aquatic media will also be a significant loss process. There is no conclusive evidence that microbial degradation is significant in aquatic media. The fate of ethylene oxide in soil will probably be similar to that in water (its fate in the atmosphere is not obvious from the available data). Available rate constants for its reaction with hydroxyl radicals and oxygen atom (^3P) and its reaction in smog chambers predict that ethylene oxide will persist in the atmosphere. No reports measuring ambient levels of ethylene oxide have been found. Only one report exists for its detection in ambient aquatic media. Several studies have detected the presence of ethylene oxide in commodities and commercial goods including food, medical supplies and drugs.

The pharmacokinetics of ethylene oxide have not been studied extensively. Only one study was found about the absorption of this chemical; it concerned the inhalation exposure of rats. The toxicity data suggest that absorption occurs via the respiratory and gastrointestinal tracts. During inhalation exposure, the highest concentration of ethylene oxide was associated with the protein fraction of the lungs, while ethylene oxide that reaches the systemic circulation is distributed widely to various tissues (liver, kidney, lung, testes, brain, spleen and intestinal mucosa). Ethylene oxide is eliminated primarily by the kidneys with the metabolite, ethylene glycol, as well as glutathione conjugates identified in the urine. Ethylene oxide also reacts with cellular macromolecules, and reaction with DNA results in small quantities of 7-hydroxyethylguanine in the urine. The half-life of ethylene oxide has been estimated to be between ≃ 10 and 30 minutes, indicating rapid removal of absorbed compound. Macromolecular-bound products and metabolites such as ethylene glycol are removed more slowly.

The effects of acute exposure of humans to ethylene oxide have been described in case reports and, to a more limited extent, in control studies. Case reports have indicated that headaches, nausea, vomiting, dyspnea and respiratory irritation occur from exposure to the vapors of ethylene oxide. Dermal contact with the liquid, aqueous solutions or clothing containing absorbed ethylene oxide results in skin burns and possibly sensitization. Control studies in humans indicate that exposure to 2200 ppm of ethylene oxide was slightly irritating, while exposure to 22,000 ppm adversely affected membranes in the nose. Studies of dermal contact indicate that 50% solutions are optimum for producing chemical burns. Some effects may be delayed several hours subsequent to exposure.

In acute studies in laboratory animals, the LC_{50} values for inhalation exposure (rats, mice and dogs) range from 835-5000 ppm for a 4-hour exposure, and the oral LD_{50} value (rabbits, guinea pigs and rats) range from 100-631 mg/kg body weight. Gross symptoms of toxicity were respiratory irritation, salivation, nausea, vomiting, diarrhea, convulsions, and death. Pathologic findings included lung, liver, and kidney damage. Ethylene oxide was an irritant in dermal studies, but failed to result in sensitization in guinea pigs. Acute effects in experimental animals appear to be similar to those reported in case reports of human exposure to ethylene oxide.

The subacute and chronic effects of ethylene oxide in man are not well-documented nor readily available from clinical case reports. Case reports of workers exposed repeatedly to ethylene oxide indicate that neurotoxicity occurred consistent with sensorimotor neuropathy. Some signs of neuropathy appeared to persist after cessation of exposure. In occupational epidemiology studies, the non-neoplastic results were reported to be an increase in lymphocyte count, a decrease in hemoglobin value (levels of exposure were not reported) and an increased incidence of deaths resulting from diseases of the circulatory system (exposure level <50 mg/m^3); however, differences in study design may account for these apparent inconsistencies. From the available data, few conclusions can be drawn regarding the systemic toxic effects of chronic exposure to ethylene oxide.

In animal studies, repeated exposures to high concentrations of ethylene oxide, 1533-643 mg/m^3, resulted in the death of rats and mice. At the lower exposure, guinea pigs and monkeys showed signs of neurotoxicity and growth depression. Decreased body weight gain was observed in rats at exposures as low as 60 mg/m^3, 6 hours/day, 5 days/week for 2 years, while signs of neurotoxicity, hunched posture and reduced locomotion were observed at doses as low as 90 mg/m^3, 5 hours/day for 10 weeks. In rats and mice, the no effect level appeared to be 18 mg/m^3. Similar signs of toxicity were observed after oral exposure to ethylene oxide, with weight loss observed at 100 mg/kg body weight and no effects observed at $<$10 mg/kg body weight (15-20 doses). Studies in animals support the observation of neurotoxicity described in human case reports.

The ability of ethylene oxide to cause teratogenic or adverse reproductive effects has been examined in a number of species (mouse, rat, rabbit, monkey and human) by two routes of administration (inhalation and intravenous). Rats, but not rabbits, exposed to 150 ppm ethylene oxide administered by inhalation displayed signs of maternal toxicity and toxicity to the developing conceptus. Ethylene oxide (150 mg/kg) administered intravenously to mice caused maternal toxicity and developmental toxicity. Intravenous administration of ethylene oxide in rabbits (9, 18 and 36 mg/kg) produced embryotoxicity associated with maternal toxicity. Ethylene chlorohydrin (ECH), a reaction product of ethylene oxide, produced adverse effects on maternal and fetal well-being in mice but not in rabbits at 120 mg/kg administered intravenously and produced adverse developmental effects without significant toxicity when administered ECH at 60 mg/kg intravenously. Ethylene oxide (100 ppm) administered by inhalation in a one-generation study caused severe adverse effects including a higher incidence of infertility, longer gestational periods, a decrease in the number of pups born, and a decrease in the number of implantation sites. The same laboratory observed lowered fetal weights, but not a substantial level of malformations in response to 100 ppm ethylene oxide administered to rats by inhalation on gestation days 6-15. Testicular degeneration was observed in hamsters and rats inhaling 204 to 357 ppm ethylene oxide. More recently, adverse effects on sperm concentration and motility but not morphology in Cynomologus monkeys exposed to 50 and 100 ppm ethylene oxide by inhalation were reported. An epidemiologic study of nursing personnel exposed to ethylene oxide found an association between ethylene oxide exposure and spontaneous abortion.

In conclusion, ethylene oxide produces adverse reproductive and teratogenic effects in both females (maternal toxicity, depression of fetal weight gain, fetal death, fetal malformation) and males (reduced sperm numbers and sperm motility) if the concentration of the chemical reaching the target organ is sufficiently high or if exposure at lower levels is sufficiently long. Thus, the experiments in which ethylene oxide was injected intravenously have produced more detrimental effects than the short-term inhalation experiments. However, even short-term inhalation experiments have resulted in suggestive evidence of detrimental effects.

Ethylene oxide has been shown to induce gene mutations in bacteria, fungi, higher plants, Drosophila, and cultured mammalian cells in tests conducted without the use of exogenous hepatic metabolic activation systems. It is therefore a direct-acting mutagen. Ethylene oxide has also been shown to induce dominant lethal effects in mice and rats; chromosomal aberrations in higher plants, Drosophila, mice, and rats; and micronuclei in mice and rats. Based on these positive findings in different test systems, ethylene oxide is judged to be capable of causing chromosomal aberrations. It has also been shown to induce sister chromatid exchange (SCE) in rabbits, rats, and humans.

Tissue distribution studies have shown that ethylene oxide reaches the gonads. This result is consistent with evidence that ethylene oxide causes unscheduled DNA synthesis (UDS) in germ cells of male mice and heritable mutations in insects and rodents (i.e., sex-linked recessive lethals and heritable translocations in Drosophila, dominant lethals in rats and mice, and heritable translocations in mice). Ethylene oxide can therefore be regarded as mutagenic both in somatic cells and in germ cells.

Based on the available data, there is overwhelming evidence that ethylene oxide is a direct-acting mutagen that has the potential to cause mutations in the cells of exposed human tissue. The observations that ethylene oxide reaches and reacts with mammalian gonadal DNA, and causes heritable mutations in intact mammals, indicates that it may be capable of causing heritable mutations in man provided that the pharmacokinetics of ethylene oxide in humans also results in its distribution to the DNA of germ cells.

Both human and experimental animal data are available to assess the carcinogenicity of ethylene oxide. The human evidence suggests an association between exposure and cancer incidence, while the animal evidence is more substantial.

Three epidemiologic studies showed a significant association between ethylene oxide exposure and the occurrence of cancer. Two of the studies found an excess risk of leukemia associated with ethylene oxide exposure.

While these studies have shortcomings and are not definitive, they do, nevertheless, constitute "limited," bordering on inadequate, epidemiologic evidence for human carcinogenicity using the U.S. Environmental Protection Agency (EPA) Proposed Guidelines for Carcinogen Risk Assessment. Two long-term inhalation studies in rats show statistically significant responses for leukemia, brain tumors and peritoneal mesothelioma. In addition, positive results for the carcinogenicity of ethylene oxide have been obtained by subcutaneous injection in mice and by intragastric administration in rats. The animal evidence for the carcinogenicity of ethylene oxide is judged to be "sufficient" using the EPA weight-of-evidence classification system.

On the basis of the human, animal, and mutagenic data cited herein, ethylene oxide is classified as being "probably carcinogenic to humans" and belonging in EPA Group B1. This classification is qualified as bordering on Group B2, however, because of limitations in the human evidence. According to the International Agency for Research on Cancer (IARC) guidelines for evaluating carcinogen evidence, ethylene oxide would be classified as Group 2A, meaning that ethylene oxide is a "probable human carcinogen," but bordering on Group 2B because of limitations in the human evidence. (See Appendix 9B for a description of the IARC classification system.)

Presuming that ethylene oxide is carcinogenic in humans, upper-limit incremental unit risk and potency estimates have been extrapolated from the 2-year rat inhalation studies. These estimates are upper limit in the sense that a true risk level cannot be pinpointed because of uncertainties in low-dose extrapolation, and, therefore, a modelling technique is employed which produces a statistical upper-bound estimate of risk while retaining biological plausibility. Upper limit means that the true risk is not likely to exceed the calculated value and may be lower. These estimates are within the range of uncertainty of those derived from the human studies. Based on leukemias and brain gliomas in rats, extrapolation to humans yields an upper-limit incremental unit risk estimate of 1.0×10^{-4}, for lifetime cancer risk resulting from continuous exposure to air that contains an ethylene oxide concentration of $1\ \mu g/m^3$. The relative potency index for ethylene oxide, which is based on both the upper-limit unit risk value and the molecular weight, is in the lower part of the third quartile of 54 chemicals that the CAG has evaluated as potential or known human carcinogens.

2. INTRODUCTION

EPA's Office of Research and Development has prepared this health assessment to serve as a "source document" for Agency use. This health assessment was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of ethylene oxide under Section 112 of the Clean Air Act. However, based on the expressed interest of other Agency offices, the scope of this document was expanded to address ethylene oxide in relation to sectors of the environment other than air. It is fully expected that this document will serve the information needs of many government agencies and private groups with health-related interests in ethylene oxide.

In the development of the assessment document, existing scientific literature has been surveyed, key studies have been evaluated and conclusions have been prepared so that the chemical's toxicity and related characteristics are identified.

The document considers all sources of ethylene oxide in the environment, the likelihood of human exposure, and the possible effect on man and lower organisms from absorption. The information found in the document is integrated into a format designed for risk assessment use. However, the information in this document on environmental levels and exposure is not intended, nor should it be used, to support any conclusions regarding risks to public health. When appropriate, the authors of the document have attempted to identify gaps in current knowledge that limit risk evaluation capabilities.

The literature searches that support this document vary somewhat. The document is current to February 1984 with the following exceptions: the mutagenicity section is current to January 1984, and the carcinogenicity section is current to January 1985.

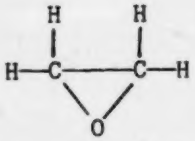
3. PHYSICAL AND CHEMICAL PROPERTIES

3.1. SYNONYMS AND CAS NUMBER

Synonyms:	1,2-epoxyethane ethylene oxide oxirane
CAS Number:	75-21-8

3.2. STRUCTURAL AND MOLECULAR FORMULAS

Structural formula:



Molecular formula:

C_2H_4O

3.3. TORSIONAL ANGLES AND BOND DISTANCES (Hirose, 1974)[a]

	Torsional Angles
<HCH	116.9
<COC	61.62
θ[b]	21.6
ϕ[c]	7.97

	Bond Distances, nm
r_m (C-C)	0.1462
r_m (C-H)	0.1086
r_m (C-O)	0.1428

[a] From microwave spectra

[b] Angle of C-C bond to H_2C plane

[c] $(<OCC)/(2-\theta)$

3.4. PHYSICAL PROPERTIES OF PURE ETHYLENE OXIDE

3.4.1. Description.

Ethylene oxide is a colorless, flammable gas which condenses at low temperatures to a colorless, clear, mobile liquid (Cawse et al., 1980; Hawley, 1981).

3.4.2. Molecular Weight.

44.05 (Weast, 1972)

3.4.3. Melting Point.

-111°C (Weast, 1972)

3.4.4. Boiling Point.

10.4°C (at 101.3 kPa = 1 atm) (Cawse et al., 1980)

3.4.5. Boiling Point Change with Pressure Change (Δbp/pressure at 100 kPa).

0.25 K/kPa (Cawse et al., 1980)

0.033 K/torr (Cawse et al., 1980)

3.4.6. Density.

d_{10}^{10}: 0.8824 (Weast, 1972)

3.4.7. Coefficient of Cubical Expansion (at 20°C, per °C).

0.00161 (Cawse et al., 1980)

3.4.8. Refractive Index (at 7°C).

1.3597 (Weast, 1972)

3.4.9. Vapor Pressure (Cawse et al., 1980).

Temperature	Vapor Pressure	
°C	kPa	Torr
-40	8.35	62.6
-30	15.05	112.9
-20	25.73	193.0
-10	42.00	315.0
0	65.82	493.7
10	99.54	746.6
20	145.8	1093
30	207.7	1558
40	288.4	2163
50	391.7	2938
60	521.2	3909
70	681.0	5108
80	875.4	6566
90	1108.7	8315
100	1385.4	10390

3.4.10. Aqueous Solubility[a] (Cawse et al., 1980).

Pressure		Temperature		
kPa	torr	5°C	10°C	20°C
20	150	45	33	20
27	202.5	60	46	29
40	300.0	105	76	49
53	397.5	162	120	74
67	502.5	240	178	101
80	600.0	NT	294	134
93	697.5	NT	NT	170
101	757.5	NT	NT	195

[a] Solubility in mℓ vapor/mℓ water, vapor volume at 0°C and 1 atm

NT = Not tested

3.4.11. Freezing Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide Weight %	Ethylene Oxide Mole %	Freezing Point °C
0	0	0.0
2.5	1.0	-0.9
5	2.1	-1.6 (eutectic)
10	4.4	5.6
15	6.7	8.9
20	9.3	10.4
30	14.9	11.1 (max)
40	21.4	10.4
50	29.0	9.3
60	38.0	7.8
70	48.8	6.0
80	62.1	3.7
90	78.6	0.0
100	100	-112.5

3.4.12. Boiling Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide Weight %	Ethylene Oxide Mole %	Boiling Point °C
0	0	100
2.5	1.0	70
5	2.1	58
10	4.4	42.5
15	6.7	38
20	9.3	32
30	14.9	27
40	21.4	21
50	29.0	19
60	38.0	16
70	48.8	15
80	62.1	13
90	78.6	12
100	100	10.4

3.4.13. Flash Point (tag open cup).

<-18°C (Cawse et al., 1980)

3.4.14. Flash Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide Weight %	Flash Point Closed Cup (°C)
1	31
3	3
5	-2

3.4.15. Explosive Limits in Air, Volume % (Cawse et al., 1980).

Upper Limit	100%
Lower Limit	3%

3.4.16. Heat of Combustion at 25°C (Cawse et al., 1980).

5.17 kJ/mol

1.24 kCal/mol

3.4.17. Log Octanol/Water Partition Coefficient.

-0.30 (Hansch and Leo, 1979)

3.4.18. Ultraviolet Spectroscopic Data (Weast, 1972).

$\lambda_{max}^{gas} = 169$ nm

log $\epsilon = 3.58$

$\lambda_{max}^{gas} = 171$ nm

log $\epsilon = 3.57$

3.5. PHYSICAL PROPERTIES AND DESCRIPTION OF COMMERCIAL ETHYLENE OXIDE

The physical properties and specifications for commercial ethylene oxide are described in Table 3-1.

TABLE 3-1

Manufacturers' Specifications for Ethylene Oxide[a,b]

	BASF	Celanese	Dow	Jefferson	Shell	Wyandotte
Purity, wt % min	99.95	99.95	NA	NA	NA	NA
Water, wt % max	0.005	0.02	0.03	0.03	0.03	NA
Aldehydes, as acetaldehyde, wt % max	0.005	0.01	0.005	0.025	0.010	0.003
Acidity, as acetic acid, wt % max	0.002	0.002	0.002	0.005	0.0020	0.002
CO_2, wt % max	0.005	NA	0.002	NA	NA	0.005
Total Cl as Cl^-, wt % max	0.005	NA	0.005	nil	NA	0.0005
Nonvolatile residue, g/100 mℓ, max	0.010	0.01	0.01[c]	0.01	0.010	0.01
Color, APHA, max	10	10	5	NA	10	10
Residual Odor	NA	none	NA	none	none	mild
Appearance	NA	clear	NA	clear	clear	NA
Acetylene, max	NA	NA	0.0005	nil	NA	NA

[a] Source: U.S. EPA, 1980

[b] This information was obtained from the respective manufacturer's product data sheets, available from each manufacturer on request.

[c] Presently, 0.005 g/100 mℓ in Dow ethylene oxide (Kurginski, 1979)

NA = Not available; wt = weight; max = maximum; min = minimum

Commercial grade ethylene oxide has a purity >99.9%. Specific impurities include trace quantities of water, aldehydes (specified as acetaldehyde), acid (specified as acetic acid), chloride, and an unspecified residue. Since commercial grade ethylene oxide is virtually pure, its physical properties are the same as those described above for pure ethylene oxide.

3.6. CHEMICAL PROPERTIES

The majority of information contained in this section was taken from Cawse et al. (1980).

Ethylene oxide is a highly reactive epoxide. Industrially, it is used principally as an intermediate for a wide variety of compounds. Most of its reactions involve opening the epoxide ring. An exception is the formation of oxonium salts with strong anhydrous mineral acids.

3.6.1. Reduction. Catalytic hydrogenation or chemical reduction of ethylene oxide results in the formation of ethanol.

3.6.2. Clathrate Formation. Ethylene oxide and water form a stable clathrate containing 6.38-6.80 molecules of ethylene oxide to 46 units of water in the unit cell. The maximum observed melting point for these compounds is 11.1°C (Section 3.4.11).

3.6.3. Polymerization. Low molecular weight polymers can be formed by the reaction of ethylene oxide and water or alcohols. The average molecular weight of these polymers (polyethylene glycols) ranges from 200-14,000, depending upon the reaction conditions. High polymers, with molecular weights ranging from 90,000 to 4 x 10^6, are formed by coordinate anionic polymerization. This reaction involves the coordination of a metallic compound with ethylene oxide to initiate the reaction. Numerous organometallic and alkaline earth compounds and mixtures are used as catalysts. This process is important in the formation of non-volatile residues during ethylene oxide storage (Section 3.5). The main catalyst for this process is rust, and no inhibitor has been found.

3.6.4. Other Reactions. Table 3-2 lists some other representative reactions of ethylene oxide.

3.6.5. Hydrolysis and Related Reactions. Epoxides degrade in water by hydrolysis and related ionic reactions and, possibly, by radical oxidations. The hydrolysis chemistry involves cleaving a carbon-oxygen bond of the cyclic ether to form ethylene glycol. Bronsted et al. (1929) noted the pathways for ethylene oxide hydrolysis in aqueous hydrochloric acid, describing hydrolysis as a combination of a noncatalytic reaction (herein referred to as the spontaneous hydrolysis) and an acid-catalyzed hydrolysis. Reaction with chloride ion was similar to hydrolysis in that chloride and epoxide reacted without catalysis and with acid catalysis.

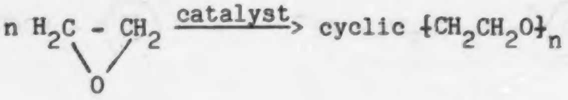
Long and Pritchard (1956) demonstrated that epoxide hydrolysis was also base catalyzed. For any epoxide, the degradation pathways are as follows for the neutral (I), acid-catalyzed (II), and alkali-catalyzed hydrolyses (III):

(I) $C_2H_4O + H_2O \xrightarrow{k_N} HOCH_2CH_2OH$ (II) $C_2H_4O + H_2O \xrightarrow[H_3O^+]{k_A} HOCH_2CH_2OH$

TABLE 3-2
Typical Reactions of Ethylene Oxide

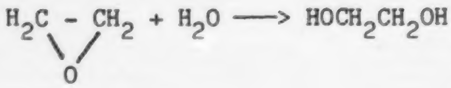
---

1. Crown Ethers



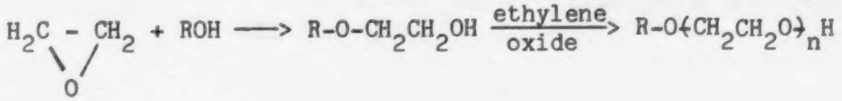
$$n\ \underset{O}{H_2C - CH_2} \xrightarrow{\text{catalyst}} \text{cyclic } \{CH_2CH_2O\}_n$$

2. Hydrolysis



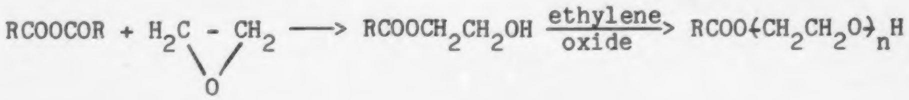
$$\underset{O}{H_2C - CH_2} + H_2O \longrightarrow HOCH_2CH_2OH$$

3. Reaction with Alcohols



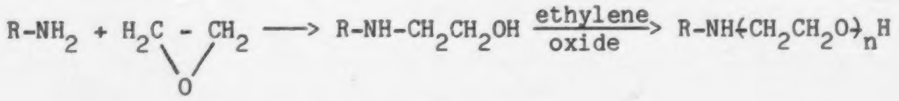
$$\underset{O}{H_2C - CH_2} + ROH \longrightarrow R\text{-}O\text{-}CH_2CH_2OH \xrightarrow[\text{oxide}]{\text{ethylene}} R\text{-}O\{CH_2CH_2O\}_nH$$

4. Reaction with Organic Acids and Acid Anhydrides



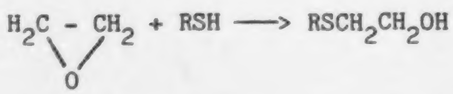
$$RCOOCOR + \underset{O}{H_2C - CH_2} \longrightarrow RCOOCH_2CH_2OH \xrightarrow[\text{oxide}]{\text{ethylene}} RCOO\{CH_2CH_2O\}_nH$$

5. Reaction with Ammonia and Primary and Secondary Amines



$$R\text{-}NH_2 + \underset{O}{H_2C - CH_2} \longrightarrow R\text{-}NH\text{-}CH_2CH_2OH \xrightarrow[\text{oxide}]{\text{ethylene}} R\text{-}NH\{CH_2CH_2O\}_nH$$

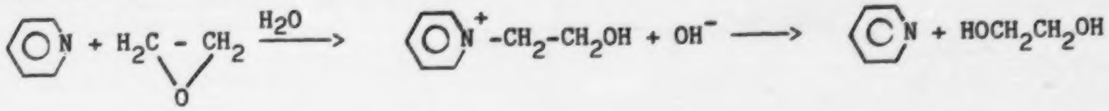
6. With Hydrogen Sulfide and Mercaptans (e.g., glutathione, cystine)



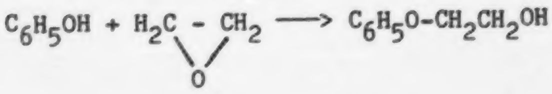
$$\underset{O}{H_2C - CH_2} + RSH \longrightarrow RSCH_2CH_2OH$$

TABLE 3-2 (cont.)

7. Reaction with Pyridine (and possibly other nitrogen heterocycles)

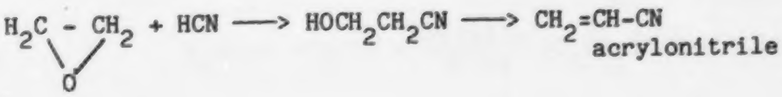

N
+ H2C - CH2
O
H2O
N+ -CH2-CH2OH + OH−
N + HOCH2CH2OH

8. With Phenols



$$C_6H_5OH + H_2C - CH_2 \longrightarrow C_6H_5O\text{-}CH_2CH_2OH$$

9. With Hydrogen Cyanide



$$H_2C - CH_2 + HCN \longrightarrow HOCH_2CH_2CN \longrightarrow CH_2{=}CH\text{-}CN$$

acrylonitrile

(III) $C_2H_4O + H_2O \xrightarrow[OH^-]{k_B} HOCH_2CH_2OH$

Table 3-3 summarizes hydrolysis data for ethylene oxide. The temperature coefficients for the rate constants are the following:

$\log k_A = 10.753 + \log T - 0.0255/R - 79.5/RT$ (Long et al., 1957)
$\log k_N = 7.726 - 79.5/RT$ (Lichtenstein and Twigg, 1948)
$\log k_B = 9.312 - 75.3/RT$ (Lichtenstein and Twigg, 1948)

Epoxides can also react with nucleophiles (anions or Lewis bases). The chemistry, although similar to hydrolysis, is more complex. The epoxide ring can be cleaved by spontaneous reaction or by acid-catalyzed reaction:

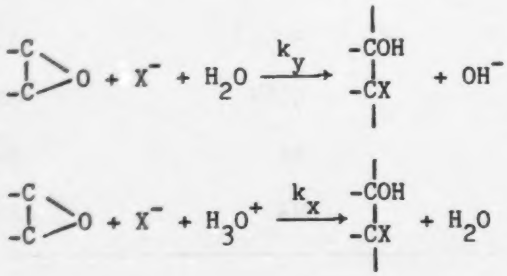


Table 3-4 summarizes specific rate constants for reactions of ethylene oxide with various anions. The consensus is that the spontaneous reaction is S_N2, but disagreement exists over whether the acid-catalyzed epoxide ring opening is A1-like or A2-like (Long et al., 1957; Lamaty et al., 1975; Pritchard and Long, 1956; Pritchard and Siddiqui, 1973; Virtanen and Kuokkanen, 1973). A discussion of the mechanism is beyond the scope of this review.

TABLE 3-3

Hydrolysis Kinetics of Ethylene Oxide

	Specific Rate Constants		
Temperature (K)	$k_A \times 10^3$ $(M^{-1}S^{-1})$	$k_N \times 10^7$ (S^{-1})	$k_B \times 10^4$ $(M^{-1}S^{-1})$
293	5.34[a]	3.61[a]	NR
293.2	NR	4.2[b]	0.65[b]
298	9.3[c]	6.75[c]	1.0[d]
298	NR	5.62[f,g]	NR
298	NR	6.17[f,h]	NR
298	NR	6.61[f,i]	NR
298	9	5.56[j]	1.1
298	NR	5.8[k]	NR
NR	10.0[d]	NR	NR
303.2	16.9[e]	NR	NR

[a] Bronsted et al., 1929

[b] Lichtenstein and Twigg, 1948

[c] Eastham and Latremouille, 1952

[d] Pritchard and Long, 1956

[e] Long et al., 1957

[f] Conway et al., 1983

[g] River water pH 7.4

[h] Sterile river water pH 7.4

[i] Sterile distilled water

[j] Long and Pritchard, 1956

[k] Koskikallio and Whalley, 1959

NR = Not reported

TABLE 3-4

Specific Rates of Reaction of Anions and Lewis Bases with Ethylene Oxide

Lewis Base or Anion	Temperature K	$10^6 k_y$ (ℓ/mol-sec)[a]	$10^2 k_x$ (ℓ^2/mol^2-sec)[a]
Cl^-	293	NR	2.17 (water)[b]
	298	NR	3.67 (water)[b]
	298	0.305[e]	NR
	300	NR	8.23 (50% aqueous ethanol)[c]
Br^-	293	NR	8.67 (water)[b]
	298	NR	14.5 (water)[b]
Pyridine	291	200 (water)[d]	NR

[a] k_y = neutral reaction; k_x = acid catalyzed

[b] Bronsted et al., 1929

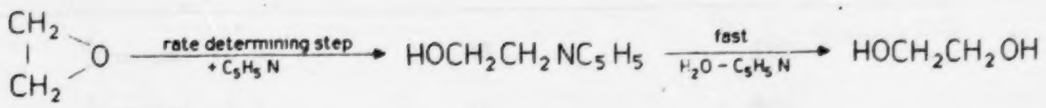
[c] Lamaty et al., 1975

[d] Pritchard and Siddiqui, 1973

[e] Conway et al., 1983

NR = Not reported

Some products of epoxide reaction with Lewis bases or with anions are not stable. For example, tertiary amines, such as pyridine, are capable of catalyzing epoxide hydrolysis to glycol:



Aqueous chemical degradation in the environment can be estimated from the contributions of hydrolysis (Equation 1) and anion reactions (Equation 2):

$$\frac{-dC_{epox}}{dt} = (k_N + k_A C_{H_3O^+} + k_B C_{OH^-})C_{epox} \tag{1}$$

$$\frac{-dC_{epox}}{dt} = (k_{yi} C_{Ai} + k_{xi} C_{Ai} C_{H_3O^+})C_{epox} \tag{2}$$

where C_{Ai}, k_{yi}, and k_{xi} refer to the concentration and specific rate constants for each anion or Lewis base. The overall degradation rate is the sum of all contributions, as given in Equation 3:

$$-\frac{dC_{epox}}{dt} = [k_N + k_A C_{H_3O^+} + k_B\, C_{OH^-} + \Sigma(k_{yi} + k_{xi} C_{H_3O^+})C_{Ai}]C_{epox} \tag{3}$$

The relative importance of chemical hydrolysis vs. reaction with chloride ion was assessed for ethylene oxide. Degradation half-lives and product distributions (chlorohydrin-to-glycol ratios) were estimated for freshwater and marine water (NaCl concentration of 3% or 0.513 M). The following specific rate constants from Tables 3-3 and 3-4 were used:

k_N $0.661 \times 10^{-6}\ s^{-1}$
k_A $9 \times 10^{-3}\ M^{-1}\ s^{-1}$
k_B $1 \times 10^{-4}\ M^{-1}\ s^{-1}$
k_{yC1} $0.305 \times 10^{-6}\ M^{-1}\ s^{-1}$
k_{xC1} $3.67 \times 10^{-2}\ M^{-2}\ s^{-2}$

Estimates were calculated for pH 5.0, 7.0, and 9.0, which is approximately the pH range of natural waters. Half-lives for chemical degradation and the chlorohydrin/glycol ratios (for sea water reactions) are summarized below:

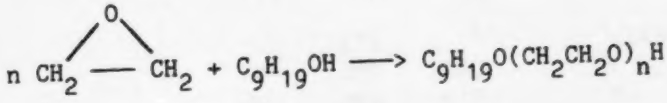
Calculated Ethylene Oxide
Half-Life at 298 K
(hours)

pH	5	7	9
Freshwater	256	291	291
Saline Solution			
0.85% (physiological)		273	
1%	240	270	270
3% (marine)	212	236	236

Conway et al. (1983) used buffered (pH 7) sterile solutions of 0, 1, and 3% NaCl to hydrolyze ethylene oxide and reported half-lives of 314, 265, and 224 hours, respectively. The half-lives for river water (pH 7.4), sterile river water (pH 7.4), and sterile distilled water were 341, 310, and 293 hours. The chlorohydrin/glycol ratios experimentally determined by Conway et al. (1983) were 0.11 and 0.23 for 1 and 3% saline solutions.

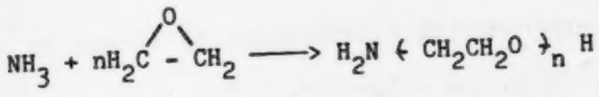
These data provide some understanding of the fate of ethylene oxide in biological fluids. The hydrolysis half-life in physiological saline (0.85% NaCl) is 273 hours or 11.4 days. This long a half-life would clearly allow for other reactions to take place. As an example, the half-life for the ethylene oxide reaction with pyridine in water is 58 minutes. Other nucleophiles present in biological systems (e.g., RS^-, $PhNH_2$) are known to be more nucleophilic than pyridine and may react with ethylene oxide much more rapidly than water or chloride.

Hydrolysis or hydrolysis-type reactions are the most significant industrial reactions of ethylene oxide. Ethylene glycol is the hydrolysis product; higher glycols (diethylene, triethylene, and polyethylene glycols) and glycol ethers result from the reaction of ethylene oxide with glycols and alcohols, respectively. Glycol esters of carboxylic acids and phenols, and ethers of cellulose, starch, and other polyols are also prepared through hydrolysis-like reactions. For example, reaction of ethylene oxide and nonylphenol yields nonylphenoxypolyethoxyethanol, a non-ionic, surface-active agent (Blackford, 1976a).



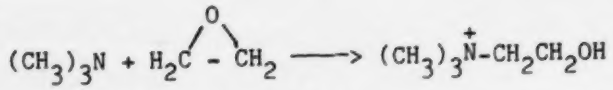
$$n\ \overset{O}{CH_2 — CH_2} + C_9H_{19}OH \longrightarrow C_9H_{19}O(CH_2CH_2O)_nH$$

Ethylene oxide reacts with amines by pathways similar to reactions with hydroxyl compounds. Reaction of ethylene oxide and ammonia yields the commercially important ethanolamines:



$$NH_3 + nH_2\overset{O}{C - CH_2} \longrightarrow H_2N \leftarrow CH_2CH_2O \rightarrow_n H$$

where n is typically 1 to 4. Choline is prepared by reacting trimethylamine with ethylene oxide (Jukes, 1964):



$$(CH_3)_3N + H_2C(O)CH_2 \longrightarrow (CH_3)_3\overset{+}{N}\text{-}CH_2CH_2OH$$

Some ionic reactions of ethylene oxide are listed in Table 3-2.

3.6.6. Free Radical Reactions. The free-radical chemistry of ethylene oxide is of particular importance in determining its fate in the atmosphere. The most important free-radical reaction is the reaction with hydroxyl radical.

Only one study was found in the available literature. Fritz et al. (1982) reported the results of a study utilizing a laser photolysis/resonance fluorescence unit designed to study the reactions of OH radicals with anthropogenic pollutants. They generated hydroxyl radical by HNO_3 photolysis and studied the reaction at 297, 377, and 435 K, at 10 torr (Ar). The following relations were reported (mean $\pm\ 3\delta$):

$$k_{(297\ K)} = (8.0 \pm 1.6) \times 10^{-14}\ cm^3/molec\text{-}sec$$

and

$$k_{(T)} = (1.1 \pm 0.4) \times 10^{-11}\ exp\ (-1460/T)\ cm^3/molec\text{-}sec$$

The mechanism involved hydrogen abstraction followed by ring opening, reaction with oxygen, nitrogen oxide and finally decomposition to carbon monoxide and formaldehyde. Ring opening may take place either before oxygen addition or after NO reaction.

4. SAMPLING AND ANALYTICAL METHODS

4.1. SAMPLING

The state-of-the-art in air sampling utilizes solid sorbents. Samples can subsequently desorb by solvent or thermal means. Critical factors in the method are the capacity of the sorbent to retain the epoxide during the collection and the complete desorption of the epoxide.

Brown and Purnell (1979) evaluated Tenax GC sampling tubes for use in ambient air monitoring studies and found them to be inappropriate for ethylene oxide. Although most of the 71 compounds tested were adequately retained, ethylene oxide was not, having the third poorest retention.

Pellizzari et al. (1976) evaluated Tenax GC and other sorbents for sampling atmospheric propylene oxide, a compound chemically very similar to ethylene oxide. Table 4-1 compares the breakthrough volumes for several sorbents. The effect of humidity on the breakthrough volume was tested for Tenax GC: breakthrough volume remained unchanged in the range of 4.0-4.5 ℓ/g when humidity was increased from 41-92%. Storage time affected the recovery of diepoxybutane (300 ng) from Tenax GC cartridges (desorbed thermally and analyzed by GC. When analysis was immediate, recovery was 100%. After the loaded cartridge was stored for 1 week, recovery dropped to 76%. Combined transport (6 days) and storage yielded recoveries of 75 and 64% after 1 and 2 weeks, respectively. Since Brown and Purnell (1979) and Pellizzari et al. (1976) used comparable methods for determining the breakthrough volume, it appears that propylene oxide and ethylene oxide behave similarly. Brown and Purnell (1979) have noted that, under the conditions of the test (5-600

TABLE 4-1

Breakthrough and Safe Sampling Volumes for Propylene Oxide with Several Sorbents

Sorbent	Breakthrough Volume ℓ/g (sorbent)[a]	Safe Sampling Volume (ℓ/g)[b]
PBL Carbon	36	9
PCB Carbon	40	10
SAL9190	40	10
MI808	24	6
Tenax GC (35/60)[c]	4	1
Porapak Q (100/120)	4	1
Chromosorb 101 (60/80)	4	1
Chromosorb 102 (60/80)	8	2
Chromosorb 104 (60/80)	>36	9

[a] Pellizzari et al., 1976

[b] Brown and Purnell, 1979

[c] Mesh size

mℓ/minute flow rate, <100 ppm vapor concentration, <20°C, and <95% relative humidity) the breakthrough volume is not less than 50% of the retention volume, and a safe sampling volume is 50% of the retention volume. If propylene oxide behavior is analogous to ethylene oxide, the reported levels of ethylene oxide present in air could not be determined accurately, since the great majority of monitoring studies use air samples larger than the breakthrough volume for ethylene oxide.

The National Institute for Occupational Safety and Health (NIOSH) has published standard procedures for ethylene oxide collection in air (NIOSH, 1977). Their procedure calls for the sampling of 5 ℓ of air through glass tubes packed with activated coconut-shell charcoal. For ethylene oxide, two tubes mounted in series are used, containing 400 and 200 mg of charcoal. If the back-up tube (200 mg) contains >25% of the epoxide, the analysis is not considered valid. Ethylene oxide should be desorbed from the charcoal with 2.0 mℓ of carbon disulfide; aliquots are then analyzed by GC with flame-ionization detection. In NIOSH (1977) tests on the analytical parameters, ethylene oxide was sampled at concentrations from 41-176 mg/m^3 (23-98 ppm); precision ($\overline{CV}_T$) was 0.103 (or standard deviation of 9.3 mg/m^3), and accuracy was 0.9% lower than the "true" value. NIOSH (1977) recommended this method for industrial hygiene monitoring at sample concentrations of 20-270 mg/m^3.

The monitoring of occupational exposure to ethylene oxide by adsorption through activated carbon and subsequent desorption with carbon disulfide was reported by Qazi and Ketcham (1977). These investigators evaluated several carbon and noncarbon adsorbants and concluded that Columbia JXC activated carbon was most suitable for the collection of ethylene oxide in air. The breakthrough volume for ethylene oxide with this adsorbant was dependent on both the flow rate and the moisture content of the air. At relative humidities >60% and sampling rates of 20-25 mℓ/minute, the breakthrough volume was <10 ℓ. The quantification of ethylene oxide was done by GC with a TERGITOL TMN or UCON LB550X column interfaced with flame-ionization detector. At concentration levels of 0.5-5.0 ppm, the average recovery of ethylene oxide by this method was 97%, with a relative standard deviation and error of 3.8 and 2.9%, respectively. The lower detection limit of the method was 0.15 ppm with a sample volume of 10 ℓ.

The quantification of ethylene oxide and its two volatile metabolites, 2-chloroethanol and ethylene glycol, at a concentration level of 1-10 ppb in biological samples was attempted by EOIC (1984). A purge and trap method consisting of nitrogen gas bubbling through purge cells, and charcoal traps for collecting the transferred volatiles was found most suitable for biological samples. The subsequent quantification of ethylene oxide and its metabolites was done by thermal desorption of the charcoal trap and analysis on interfaced GC/MS operated on a selected ion-monitoring mode. Both packed carbowax 20 M and Earbopack/THEED GC columns were used; however, this technique provided non-reproducible data due to deterioration (peak tailing) of the GC columns with time.

Romano and Renner (1975) described the results of a six-laboratory inter-comparison of three methods for sampling ethylene oxide concentrations in surgical equipment. The study was administered through a Subcommittee on Ethylene Oxide Sterilization of the Association for Advancement of Medical Instrumentation. The three sampling methods were vacuum extraction with sample freezeout, headspace analysis, and acetone extraction. The vacuum-freezeout technique requires distillation of volatiles from the sample, and freezing them in a cold trap. The sample is then vaporized and an aliquot is removed with a vacuum syringe for GC analysis. This method requires greater time and equipment than the other techniques and is subject to errors from equipment leaks; however, it is the most sensitive method, and, since the sample injected into the GC is a vapor, column life is prolonged. Acetone extraction consists of partitioning the epoxide between the sample and the acetone solvent. Its advantage is its simplicity. Its disadvantages include its inability to quantitatively extract epoxide, impurities from the solvent and the plastics, the reduced lifetime of GC columns, and low sensitivity. In headspace analysis, the sample is placed into a vial equipped with a septum for gas withdrawal by syringe. The epoxide partitions between the sample and headspace gases. Romano et al. (1973) reported that the headspace technique has a lower limit of 0.1 ppm and that the technique can be automated. The advantages of this technique include its ease of performance, speed, sensitivity, and relatively long column life. Its disadvantage is that leaks in septa, vial caps, etc., can yield low measurements. Among the three overall methods, Romano and Renner (1975) found no significant differences, though they did find slight differences between laboratories.

Ben-Yehoshua et al. (1971) analyzed fruit pulp by blending it with 50 mℓ of analytical grade acetone for 30 seconds and filtering the homogenate to clarity. The samples were then stored at -10°C in bottles with self-sealing stoppers. Measurements (by GC) of added ethylene oxide and its residues were accurate to ±5%. Scudamore and Heuser (1971) extracted wheat flour and other commodities, including coconut, sultanas, lentils, and ground nuts with 5:1 (v/v) analytical grade acetone:water. The extraction used as little as 3 mℓ solvent/g sample. A contact time of 24 hours was sufficient to yield ethylene oxide recoveries (by GC) of $\geq 95\%$. Pfeilsticker et al. (1975) extracted 10 g of grain (not crushed) with 5 mℓ of methanol using continuous agitation for 24 hours. Recovery of ethylene oxide was 73% (25±1.7).

Brown (1970) sampled and analyzed surgical materials (plastic and rubber) for ethylene oxide residues by means of a three column chromatography system. This system could separate ethylene oxide and its degradation product, ethylene chlorohydrin. Epoxides were extracted with p-xylene (3 days of contact) or co-sweep distillation. The p-xylene solution was passed through one column of Florisil; ethylene chlorohydrin remained fixed in the column and ethylene oxide passed through. The solution was then passed through the second acid-celite column, which converted any ethylene oxide to ethylene chlorohydrin. A third Florisil column retained the ethylene chlorohydrin, which was subsequently eluted with petroleum ether. The sample was concentrated and analyzed by GC. Brown (1970) reported values as low as 1.8 ppm, but the accuracy, precision, and minimum detection limit were not described.

4.2. ANALYSIS

To date, GC analysis for ethylene oxide has used only flame-ionization or thermal-conductivity detection. Neither detection system is selective, so the epoxides must be separated from all interfering components, and the analytical column must be chosen around potential interferences. Columns used for epoxide analysis have included uncoated Poropak Q, QS, and R, and Chromosorb 102 (Taylor, 1977a,b; Ben-Yehoshua and Krinsky, 1968; Steinberg, 1977), and a variety of coated columns. The most common liquid phases appear to be SE-30, Carbowax 20M, and polypropylene glycol (Ben-Yehoshua and Krinsky, 1968;

Casteignau and Halary, 1972; Steinberg, 1977; Hughes et al., 1959). Bertsch et al. (1974) used a 100m x 0.5mm capillary column coated with Emulphor ON 870. The GC methods in current use appear capable of epoxide analysis at the ppm level.

The EOIC (1984) used a GC/MS/SIM system for the quantification of ethylene oxide and its volatile metabolites in biological samples. More recent techniques involving a negative-ion atmospheric pressure chemical ionization MS/MS system developed by Sciex of Canada were used by U.S. EPA (1984) for the on-site monitoring of pollutants in the atmosphere. This system was used for the identification, but not for the quantification, of ethylene oxide in a synthetic gas mixture.

Other analytical methods include various wet chemical techniques. Epoxides can be analyzed by ring opening with specific reagents and subsequent analysis for that reagent or one of its products (Dobinson et al., 1969). For example, Mishmash and Meloan (1972) reported what may be the most recent use of this approach. Butylene oxide was hydrolyzed to its glycol, and the glycol was oxidized with periodic acid. Residual oxidant was analyzed by adding CdI_2-starch and measuring the starch-I_3 complex concentration at 590 nm. They claimed a detection limit in the nmole range.

5. SOURCES IN THE ENVIRONMENT

5.1. PRODUCTION

5.1.1. Quantities Produced. Production volumes and sales quantities for ethylene oxide are listed in Table 5-1 for the years 1972 to 1982.

5.1.2. Producers, Production Sites, and Distribution. The producers, production sites, and annual capacities of ethylene oxide are listed in Table 5-2. ICI Americas is building a new ethylene oxide plant in Bayport, Texas; the nameplate capacity is 520 million pounds/year (Anonymous, 1981a). Dow will add 400 million pounds/year capacity onto its Plaquemine, Louisiana, facility during the fourth quarter of 1983. Union Carbide is building a 400 million pounds/year unit in Alberta, Canada, slated to be on stream in 1985. PPG Industries and DuPont are conducting a feasibility study to determine whether to move the former's idle Guayanilla, Puerto Rico, facility (rated at 300 million pounds/year) to Beaumont, Texas, to be operated by both companies.

5.1.3. Production Methods and Processes.

5.1.3.1. INTRODUCTION -- The majority of the information in this section was obtained from Cawse et al. (1980).

Ethylene oxide is produced almost exclusively by direct oxidation, using either air or oxygen. Other processes cannot compete with the lower operating costs of direct oxidation. Only one plant in the United States currently has

TABLE 5-1

Ethylene Oxide Production[a,b]

Year	Production	Sales[c]
1982[d]	5200 (2359)	NA
1981	4937 (2240)	NA
1980	5220 (2368)	531 (241)
1979	5665 (2570)	560 (254)
1978	5012 (2273)	525 (238)
1977	4364 (1980)	549 (249)
1976	4184 (1898)	439 (199)
1975	4467 (2026)	409 (186)
1974	3893 (1766)	457 (207)
1973	4167 (1890)	501 (227)
1972	3962 (1797)	454 (206)

[a] Source: USTC, 1974, 1975; USITC, 1976, 1977a, 1977b, 1978, 1979, 1980, 1981

[b] All quantities are expressed in millions of pounds; SI units in millions of kilograms are given in parentheses.

[c] The difference between production and sales does not enter the merchant marketplace.

[d] Projected (Source: Anonymous, 1982)

NA = Not available

TABLE 5-2

Ethylene Oxide Producers, Plant Sites, Capacities, Processes, and Technology[a]

Company	Location	Annual Capacity[b]	Process Oxidant	Technology
BASF Wyandotte, Indust. Chem. Group Basic Chems. Div.	Geismar, LA	481 (216)	oxygen	Shell
Calcasieu Chem. Corp.[c]	Lake Charles, LA	225 (101)	oxygen	Shell
Celanese Corp. Celanese Chem. Co., Inc.	Clear Lake, TX	425 (191)	oxygen	Shell
Dow Chemical U.S.A.	Freeport, TX	260[d] (117)	air	Dow
	Plaquemine, LA	450[e] (203)	air	Dow
Eastman Kodak Co. Eastman Chemical Prod., Inc. Subsid. Texas Eastman Co.	Longview, TX	195 (88)	oxygen	Shell
ICI Americas, Inc., Petrochems. Div.	Bayport, TX	520 (234)[f]	NA	NA
Inter-North, Inc. Northern Petrochem. Co., Subsid. Petrochems. Div.	Joliet, IL	230 (104)	oxygen	Scientific Design
Olin Corp., Olin Chems. Group	Brandenburg, KY	110 (50)	oxygen	Shell
PPG Industries, Inc. Chems. Group, Chem. Div.-U.S.	Beaumont, TX	155 (70)	air	Scientific Design

TABLE 5-2 (cont.)

Company	Location	Annual Capacity[b]	Process Oxidant	Technology
Shell Chemical Co.	Geismar, LA	700 (315)	oxygen	Shell
Sun Olin Chemical Co.	Claymont, DE	100 (45)	oxygen	Shell
Texaco, Inc. Texaco Chemical Co., Div.	Port Neches, TX	700 (315)	air	Scientific Design
Union Carbide Corp. Chems. and Plastics Div.	Seadrift, TX	1000 (450)	air	Union Carbide
	Taft, LA	1250 (563)	air	Union Carbide
Union Carbide Carbie, Inc., Subsid.	Ponce, PR	640 (288)	air	Union Carbide

[a] Sources: Anonymous, 1981a; SRI International, 1981a,b; Cawse, 1980

[b] Capacities are expressed in millions of pounds; capacities in millions of kilograms are in parentheses.

[c] Plant is on indefinite standby as of January 31, 1981 (Anonymous, 1981a).

[d] Approximately 200 million pounds/year (90 million kg/year) additional capacity can be obtained from a chlorohydrin unit used for propylene oxide production.

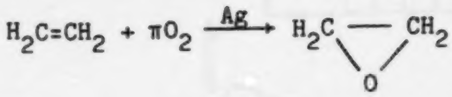
[e] Expansion of 400 million pounds/year (180 million kg/year) is due in the fourth quarter of 1983.

[f] Under construction

NA = Not available

chlorohydrin capacity (Dow at Freeport, Texas; see Table 5-2). The major drawback of the direct oxidation process is the loss of ≈25-30% of the ethylene to carbon dioxide and water.

5.1.3.2. DIRECT OXIDATION -- The overall reaction for direct oxidation can be represented as follows:



5.1.3.2.1. Air-Based Oxidation -- The schematic for air-based ethylene oxidation is presented in Figure 5-1. Little detailed information is available concerning process technology; however, the salient features are presented below.

In the first section, air and ethylene are fed into the recycle gas stream (the recycle gas contains unreacted starting material from the main absorber). The recycle stream is fed into a bank of tubular main reactors, the number of reactors depending chiefly on the capacity of the plant, activity of the catalyst, and size of the reactors. In the main reactor, the ethylene is oxidized to ethylene oxide, carbon dioxide, and water, as well as to minor components such as formaldehyde and acetaldehyde.

Ethylene conversion to ethylene oxide per pass in the main reactors is 20-50%. Oxidation inhibitors (e.g., vinyl chloride, ethylene dichloride) are added to retard carbon dioxide formation. The process stream leaving the reactor may contain 1-2 mole % ethylene oxide. This hot effluent gas is cooled to around 35-40°C and fed to the main absorber.

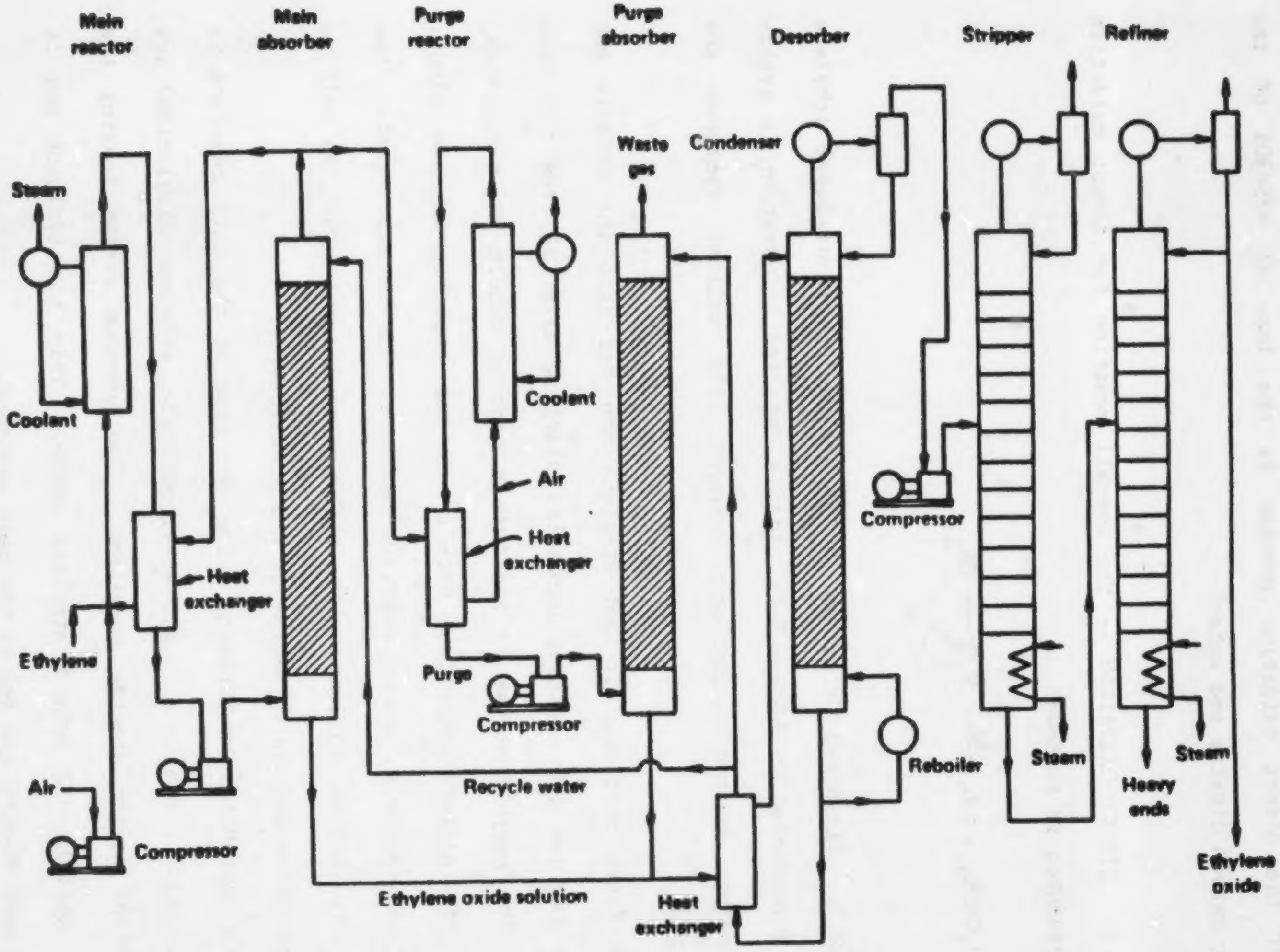

Main reactor
Main absorber
Purge reactor
Purge absorber
Desorber
Stripper
Refiner
Steam
Coolant
Ethylene
Air
Compressor
Heat exchanger
Coolant
Air
Heat exchanger
Purge
Compressor
Waste gas
Condenser
Compressor
Recycle water
Ethylene oxide solution
Heat exchanger
Reboiler
Steam
Heavy ends
Steam
Ethylene oxide

FIGURE 5-1

Schematic for Air-Based Ethylene Oxidation

Source: Schultze, 1965

The main absorber uses cold water to dissolve the ethylene oxide, some carbon dioxide, and traces of hydrocarbons and aldehydes. The unabsorbed gas is split overhead. The largest portion is used as recycle gas, and to cool the effluent stream from the main reactor; the gas then enters the main reactor. A much smaller portion of the absorber effluent gas is fed as the main stream to the secondary or purge reactor. The effluent from the purge reactor is heat exchanged with the main stream and sent to the purge absorber which operates in the same manner as the main absorber.

The purge reactor system reacts a large portion of the ethylene present in the purge gas from the main reactor which must be vented from the main reactor so that inert gases (principally nitrogen and carbon dioxide) do not accumulate. Although Figure 5-1 shows a two stage air-based plant with a single purge reactor, some large plants have three or more stages to improve the overall yield. These plants merely place another purge reactor and absorber in series.

In some plants, the ethylene content of the vent gas is sufficiently high to make energy recovery economical. This not only produces valuable power from the vent gas, but also reduces the hydrocarbon emissions from the process.

The remainder of the process involves purification. The ethylene oxide-water solution from the absorbers is heat-exchanged and sent to the desorber, where the ethylene oxide is steam stripped under reduced pressure. The ethylene oxide is collected at the top and compressed for further purification, while the stripped water is recirculated to the main and purge absorbers.

The ethylene oxide from the desorber still contains some carbon dioxide, nitrogen, aldehydes, and traces of ethylene and ethane, and must be sent to the stripper. Here, the light gases are separated overhead and vented, while the partially purified ethylene oxide is taken from the bottom of the stripper and sent to the mid-section of a final refining column. The ethylene oxide from the refining section should have a >99.5 mole-% purity.

The specific conditions used to operate ethylene oxide plants are proprietary information; however, the general ranges suggested by the literature and patent reviews have been summarized by Cawse et al. (1980) and are presented in Table 5-3.

5.1.3.2.2. Oxygen-Based Oxidation -- The differences in oxygen-based and air-based oxidation processes are almost entirely the result of the change in oxidants. The main difference is that the purge reactor is absent in the oxygen-based process and a carbon dioxide removal unit and an argon vent are added. In the air-based cycle, the low per-pass conversion, the necessity of complete ethylene oxide removal in the absorber, and the accumulation of nitrogen necessitates a substantial purge system. Because of this, a staged reaction-absorption system is required. Since the oxygen-based process uses essentially pure oxygen, the recycle gas is almost entirely unconverted ethylene; hence, there is no need for a purge system. Carbon dioxide, however, is still produced in the oxygen system, and because it has a negative effect on catalyst selectivity, the carbon dioxide must be removed. In addition to the carbon dioxide removal unit, an argon vent is required. Argon is a major impurity in oxygen and can build up to the extent of 30-40 mole-%.

TABLE 5-3

Ranges of Reaction System Variables in the Direct Air-Oxidation of Ethylene Oxide[a]

Variable	Range
ethylene, mole %	2-10
oxygen, mole %	4-8
carbon dioxide, mole %	5-10
ethane, mole %	0-1.0
temperature, °C	220-277
pressure, MPa (psi)	1-3 (145-435)
space velocity[b], h^{-1}	2000-4500
pressure drop, kPa (torr)	41-152 (308-1140)
conversion, %	20-65
selectivity or yield (mole basis, %)	63-75

[a] Source: Cawse et al., 1980

[b] The space velocity is the standard volume of the reactant stream fed per unit time divided by the volume of reactor space filled with catalyst.

h = hour

In spite of this additional purge, the total vent stream from an oxygen-based plant is much smaller than that of an air-based plant.

As is the case with an air-based unit, the main process vent stream usually contains a high concentration of hydrocarbons. In such a case, the purge stream can be used for energy recovery. The operating ranges for an oxygen-based process are summarized in Table 5-4.

The choice of oxygen versus air as the oxidant is based strictly on economics; in general, for small-to-medium capacity units (<50,000 tons/year), oxygen-based plants have lower capital cost even with the necessary air separation facility. For medium-to-large plants (75,000-150,000 tons/year), the air process investment is smaller unless oxygen can be purchased from a very large air separation facility. Operating costs of the facilities can differ significantly and are based on the cost of ethylene, oxygen, catalyst, and energy.

5.1.3.2.3. Chlorohydrin Processes -- The chlorohydrin process was the main method of ethylene oxide manufacture until 1957. In 1972, the Dow Chemical Company converted the remaining chlorohydrin capacity plants to the production of propylene oxide, and the process was not used again for ethylene oxide production until 1975. The Dow Chemical Company has built-in flexibility for using the chlorohydrin process to produce either propylene oxide or ethylene oxide. Since 1975, part of this capacity has been used for ethylene oxide. During 1975, the Dow Chemical Company made between 25 and 50 million pounds of ethylene oxide via the chlorohydrin process (Blackford, 1976b). The chlorohydrin process is attractive commercially only when a good supply of captive low-cost chlorine and lime or caustic soda is available.

TABLE 5-4

Ranges of Reaction System Variables in the Direct Oxygen-Oxidation of Ethylene Oxide[a]

Variable	Range
ethylene, mole %	15-40
oxygen, mole %	5-8.5
carbon dioxide, mole %	5-15
ethane, mole %	0-2
argon, mole %	5-15
nitrogen, mole %	2-60
methane, mole %	1-60
temperature, °C	220-275
pressure, MPa (psi)	1-2.2 (145-319)
space velocity[b], h^{-1}	2000-4000
conversion, %	7-15[c]
selectivity or yield (mole basis, %)	70-77

---

[a] Source: Cawse et al., 1980

[b] The space velocity is the standard volume of the reactant stream feed per unit time divided by the volume of reactor space filled with catalyst.

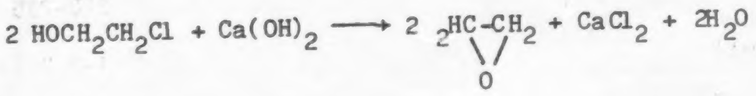
[c] At 30 mole % ethene

h = hour

Also, satisfactory markets or disposal facilities are needed for the major by-products (Schultze, 1965).

The chlorohydrin process starts with conversion of ethylene to ethylene chlorohydrin with hypochlorous acid. The chlorohydrin is converted to ethylene oxide by dehydrochlorination with slaked lime. Two major by-products, 1,2-dichloroethane (≈100-150 pounds/1000 pounds ethylene oxide) and bis(2-chloroethyl)ether (≈70-90 pounds/1000 pounds ethylene oxide), are formed during the chlorohydrin formation; acetaldehyde (5-10 pounds/1000 pounds ethylene oxide) is produced during the dehydrochlorination.

The formation of ethylene oxide from ethylene chlorohydrin can be represented by the following equation:



$$2\,HOCH_2CH_2Cl + Ca(OH)_2 \longrightarrow 2\;{}_2HC\text{-}CH_2 + CaCl_2 + 2H_2O$$

Ethylene chlorohydrin is formed in the lower section of a reaction tower. Gases are separated from the dilute chlorohydrin solution in the top section and the vent gases from the condensing apparatus pass in series to water and caustic scrubbers, where residual chlorine and HCl gas are removed before the unreacted ethylene is recycled. The aqueous chlorohydrin solution is mixed with a 10% solution of milk of lime at the inlet to the hydrolyzer (Schultze, 1965).

The crude ethylene oxide product from the hydrolyzer contains ≈77.5% ethylene oxide, 10% water, 12% chlorinated organic compounds [principally 1,2-dichloroethane and bis(2-chloroethyl)ether], and 0.5% acetaldehyde together with small amounts of hydrocarbon gases. This crude ethylene oxide is refined in two columns; the first column removes chlorinated hydrocarbons and the second column removes acetaldehyde.

5.2. USES OF ETHYLENE OXIDE

A description of the various uses of ethylene oxide is given below:

	Billion Pounds[a]	Percent of Total
Ethylene glycol	3.2	62
Nonionic surface-active agents	0.62	12
Glycol ethers	0.31	6
Ethanolamines	0.26	5
Miscellaneous applications (higher glycols, urethane polyols, sterilant, fumigant, export)	0.78	15

Source: Anonymous, 1981a

[a] Based on 1982 production estimates of 5.2 billion pounds.

The major users and use sites for ethylene oxide are listed in Table 5-5. As can be seen from this table, a very large percentage of production is captively consumed by the primary manufacturers. A general description of the various uses of ethylene oxide is presented below.

5.2.1. Ethylene Glycol. By far, the largest single use of ethylene oxide is its use captively as an intermediate in the synthesis of ethylene glycol, which is currently produced by hydration of ethylene oxide. Current industry capacity to produce ethylene glycol is 5815 million pounds annually (Anonymous, 1981b). The growth in consumption of ethylene oxide has largely depended on its use as an intermediate for ethylene glycol production.

TABLE 5-5

Users and Use Sites of Ethylene Oxide*

Company	Location	Ethylene Glycol	Glycol Ethers	Diethylene Glycol	Ethanol-amine	Triethylene Glycol	Polyethylene Glycol
BASF Wyandotte Corp.	Geismar, LA	+	-	+	-	-	-
	Wyandotte, MI	-	-	-	-	-	+
Calcasieu Chem.	Lake Charles, LA	+	-	-	-	-	-
Celanese Chem.	Clear Lake, TX	+	-	+	-	+	-
Dow Chem.	Freeport, TX	+	-	+	+	+	+
	Plaquemine, LA	+	-	+	-	+	-
	Midland, MI	-	+	-	+	-	-
Eastman Kodak	Longview, TX	+	+	+	-	+	-
Northern Petrochem.	Morris, IL	+	-	+	-	-	-
Olin Corp.	Brandenburg, KY	+	+	+	+	+	+
PPG Ind.	Beaumont, TX	+	+	+	-	+	-
	Guayanilla, PR	+	-	+	-	+	-
Shell Chem.	Geismar, LA	+	+	+	-	+	-
Texaco Jefferson Chem.	Port Neches, TX	+	+	+	+	+	+
Union Carbide	Seadrift, TX	+	+	+	+	+	-
	Taft, LA	+	+	+	-	+	-
	Penuelas, PR	+	+	+	-	+	-
	Texas City, TX	-	-	+	-	-	-
	Institute and S. Charleston, WV	-	-	-	-	-	+
Ashland Chem.	Janesville, WI	-	-	-	-	-	+
Hoadag Chem.	Skokie, IL	-	-	-	-	-	+

*Source: SRI International, 1977

+ indicates user of ethylene oxide; - indicates non-users of ethylene oxide

(Blackford, 1976b). Ethylene glycol is used mainly in polyester production and antifreeze formulations (Anonymous, 1981c).

5.2.2. Nonionic Surface-Active Agents. Of the nonionic surface-active agents synthesized from ethylene oxide, ≈25% are of the cyclic variety, while ≈75% are of the acyclic variety. In the cyclic group, ethylene oxide is used to make ethoxylate alkyl phenols and alkylphenol-formaldehyde condensates. Production of ethoxylated nonylphenol is probably the largest volume product of the cyclic group; another large-volume product is ethoxylated dodecylphenol. These surface-active agents are primarily used in detergents. The acyclic surface-active category includes ethylene oxide used in the synthesis of surface-active polyethylene glycol esters, ethoxylated alcohols, polyether polyols, ethoxylated fats and oils, and miscellaneous ethoxylated products, such as mercaptans, glycols, and polyols (Cogswell, 1980). Industry estimates that ethylene oxide consumption for acyclic surface-active agents is expected to increase. The manufacture of ethoxylated linear alcohols, used in heavy-duty liquid detergents, will account for most of this growth (Cogswell, 1980).

5.2.3. Di-, Tri-, and Polyethylene Glycols. Ethylene oxide and ethylene glycol react to form diethylene glycol, triethylene glycol, and polyethylene glycol. Diethylene and triethylene glycols are obtained mainly as by-products of ethylene glycol manufacture. Diethylene glycol is used to produce polyester resins, as a textile lubricant, and in solvent extraction. Triethylene glycol is used as a humectant and in natural gas dehydration, vinyl plasticizers, and polyesters. Industry capacity to make diethylene glycol is 472 million pounds/year; capacity to make triethylene glycol is ≈145 million pounds/year (SRI International, 1977).

5.2.4. Glycol Ethers. Ethylene oxide is combined with alcohols to manufacture glycol monoethers. These include ethylene glycol monomethyl, monoethyl, and monobutyl ethers and diethylene and triethylene monoethyl, monomethyl, and monobutyl ethers (Cogswell, 1980). Glycol ethers are used mainly as solvents. Industry capacity to make glycol ethers is 865 million pounds annually (SRI International, 1977).

5.2.5. Ethanolamines. Ethylene oxide reacts with ammonia to form a mixture of mono-, di-, and triethanolamines. The proportion of each of three ethanolamines is dependent upon the ratio of reactants used. About 25-30% of all ethanolamines are used in soaps and detergents, 5-20% in scrubbing acid gases (especially in the synthesis of ammonia), 10% by the metal industry, 8% by the textile industry and 5-15% in toilet goods (Blackford, 1976b). The remainder is used in various other applications.

5.2.6. Miscellaneous Applications. Ethylene oxide is consumed in the synthesis of numerous commercial chemicals. The largest amount in the miscellaneous group described above goes into the production of polyether polyols for flexible polyurethane foams. In 1978, ≈100 million pounds (45 million kg) of ethylene oxide were consumed in making these polyols (Cogswell, 1980).

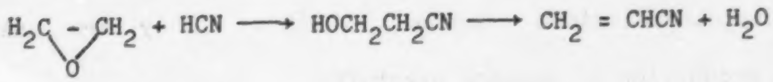
Approximately 17 million pounds of ethylene oxide is used annually to make the medicinals, choline and choline chloride; another 10 million pounds of ethylene oxide is used annually in the manufacture of hydroxyethyl starch, a semi-synthetic gum used in textile sizing and in adhesives. The production of hydroxyethyl cellulose, another adhesive additive, produced by the reaction of cellulose with ethylene oxide uses ≈25 million pounds (11 million kg) of ethylene oxide annually (Cogswell, 1980). Arylethanolamines are made by reacting ethylene oxide with either aniline or aniline derivatives. It is estimated that ≈3 million pounds (1.4 million kg) of ethylene oxide are used annually to make arylethanolamines (Cogswell, 1980). They are used as intermediates in the production of monoazo dyestuffs.

Acetal copolymer resins are produced by the catalytic copolymerization of 1,3,5-trioxane with a cyclic ether such as ethylene oxide. Ethylene oxide consumed in this use is believed to have been ≈2 to 3 million pounds/year (0.9-1.4 million kg) from 1977-1978 (Cogswell, 1980).

Ethylene oxide is used to produce ethoxylated cationic surface-active agents (non-ionic surface-active agents are discussed in Section 5.2.2). Several million pounds of ethylene oxide are used annually to produce cationic agents such as ethoxylated (coconut oil alkyl) amine, ethoxylated (tallow alkyl) amine, and various ethoxylated fatty acid amino amides (Blackford, 1976b).

Small amounts of ethylene oxide are also used as a fumigant, as a sterilant for food and cosmetics, and in hospital sterilization (Gilmour, 1978). In 1975, an estimated 0.1 million pounds of ethylene oxide were used for fumigant purposes (Landels, 1976). Dow Chemical (Kurginski, 1979) has estimated that <0.2% of production (≈10 million pounds/year) of ethylene oxide is used as a fumigant; however, the exact figure is not available.

5.2.7. Discontinued Uses of Epoxides. The only significant discontinued use of ethylene oxide known is the production of acrylonitrile. Until 1953 (when acetylene was first used), all acrylonitrile was produced by the catalytic dehydration of ethylene cyanohydrin that was prepared from ethylene oxide and hydrogen cyanide. The reaction may be represented as follows:



In 1956, American Cyanamid Company closed its 35 million pounds/year plant at Warners, New Jersey, which was based on this process. From then until 1966, when it was discontinued, this process was used only by Union Carbide at Institute, West Virginia (Blackford, 1974). In 1965, Union Carbide consumed 90 million pounds of ethylene oxide to make acrylonitrile.

5.2.8. Projected or Proposed Uses. Wood treatment is a potentially important market for epoxides (Anonymous, 1977). The USDA Forest Product Laboratory has reported that treating southern yellow pine with epoxides (including ethylene oxide, propylene oxide, and butylene oxide) improves its durability. The treatment adds 20-30% (by weight) of the epoxide to the wood.

5.2.9. Alternatives to Uses for Ethylene Oxide. More than 99% of ethylene oxide produced in the United States is used as a chemical intermediate in chemical syntheses of glycols and other compounds. Alternatives would require production routes from raw materials other than ethylene oxide.

Roughly 62% of the ethylene oxide produced is hydrolyzed to ethylene glycol. A new process for making ethylene glycol directly from ethylene has been developed by Halcon, Inc. (Klapproth, 1976). Ethylene is reacted with acetic acid in the presence of a catalyst to form mono- and diacetates, which are then hydrolyzed to ethylene glycol. Oxirane Corporation has constructed an 800 million pounds/year plant based upon this technology in Channelview, Texas. This represents ≈25% of the present total industry ethylene glycol capacity.

For other compounds synthesized from ethylene oxide, no information was available on synthesis from other raw materials.

About 0.1 million pounds of ethylene oxide are used as a fumigant annually (Dow Chemical estimates that the volume of ethylene oxide used as a fumigant is <0.2% of total production, which in 1978 would be equal to <10 million pounds; Kurginski, 1979). It seems possible that alternative commercial fumigants could replace ethylene oxide in many of its fumigant uses.

5.3. POTENTIAL FOR ENVIRONMENTAL CONTAMINATION

5.3.1. Air Emissions from Production. Air emissions from direct-oxidation ethylene oxide plants of all types consist mainly of ethylene, ethylene oxide, and traces of ethane. The main process vent stream is responsible for most of the air emissions in both air- and oxygen-based units. In air units, this vent is located on the last purge reactor absorber and is principally spent air (N_2, O_2, and some inert gases), carbon dioxide, traces of ethylene oxide, and generally <2 mole % hydrocarbons. A catalytic converter is sometimes added to the main process vent in an air system.

The analogous vent stream from an oxygen-based system is ≈100 times smaller and contains a much higher hydrocarbon concentration, and is consequently used as a fuel. Table 5-6 presents approximate concentrations of typical vent stream contaminants for the main process vent and the purge gas vent.

For unburned vent gas from an oxygen-based unit, the total hydrocarbon emissions have been estimated to be ≈12 g/kg product. If methane is used as a diluent and the purge gas incinerated, the emissions can be reduced to ≈4 g/kg product. In an air-based unit without catalytic combustion of the purge gas, hydrocarbon emissions are estimated to be >30 g/kg product. The use of a catalytic converter can reduce emissions to ≈15 g/kg product. In a study conducted for the U.S. EPA, the total ethylene oxide emissions in 1978 were estimated to be $\approx 2 \times 10^6$ pounds (9.09×10^6 kg) (Systems Application, Inc., 1982).

The major aqueous waste is draw-off from separator bottoms (Liepins et al., 1977). The process water is recycled in ethylene oxide manufacture and in the primary use of ethylene oxide as an intermediate in ethylene glycol manufacture (Sittig, 1962, 1965). The aqueous waste from direct oxidation plants will contain small amounts of glycols, aldehydes, and heavy glycols (Cawse et al., 1980). No information was available on how much of the process water eventually is treated, and no details were provided on treatment methods. The wastewater will have a high BOD, but inorganic and refractory organics appear to be minimal problems (Sittig, 1962, 1965; Spencer, 1971). Conventional water treatment (including filtration and flocculation) with a

TABLE 5-6

Typical Vent Gas Composition for Both Air- and Oxygen-Based Ethylene Oxide Plants*

Stream	Range, mole %	
	Air-Based	Oxygen-Based
Main Process Vent		
nitrogen	85-93	2-35
oxygen	1.0-5	5-7
methane	0-0.9	1-35
ethane	trace-0.2	trace-0.2
ethylene	trace-2.5	13-35
ethylene oxide	0-0.01	0-0.01
carbon dioxide	5-15	5-15
argon	NP	5-15
water	0.1-1.5	0.1-0.5
CO_2 Rich Purge Gas (water-free)		
nitrogen	13-25	NP
oxygen	1-26	0.02
ethylene and hydrocarbons	2.5-8.0	0.3-0.9
ethylene oxide	0-1.0	NP
carbon dioxide	62-80	99-99.7
inert compounds	NP	0.005-0.015

*Source: Cawse et al., 1980

NP = Not present

biological treatment appears sufficient to remove the major contaminants of the process water (Spencer, 1971; Shenderova et al., 1972). There is no solid waste produced during ethylene oxide manufacture.

5.3.2. Handling, Transport, and Storage. Ethylene oxide could be released as a result of fugitive emissions or venting during its handling, transport, or storage. No specific information was available to describe these losses; information on current practices, procedures, or environmental controls was sparse and no monitoring information was available. The potential situations of release of epoxides without attempting to establish their relative importance have been discussed in the following paragraphs.

Bulk shipments of ethylene oxide are commonly made by railroad 10,000 and 20,000 gallon freight tankers. Shipments are also made in special 55-gallon drums and by highway truck tankers. Ethylene oxide is stored in bulk containers, as well as in smaller quantities in 55-gallon drums. No information was available on the usual emission controls used on storage and transport containers. "Padded" containers, if used, would conserve vapors which would otherwise be vented to the atmosphere. Emissions could also occur during equipment purging in routine maintenance, gauge glass blowdown, or leaks.

Release is also possible during transfer. In normal practice, railway tankers are loaded and unloaded directly from or into storage tanks. The chemical is transferred under nitrogen pressurization (≈50 psi) for pumping. Faulty equipment or over-pressurization could cause epoxide emissions. Small amounts could be spilled during handling as well.

One concern in addition to normal working and handling losses is release from a storage container or transport-related accident. This could range from a relatively minor incident, such as release through a pressure safety valve or a rupture disc, to a major accident in which an entire storage container or tanker ruptures. No information was available to predict how often the minor release accidents occur or the amount of ethylene oxide they annually release.

Storage, transport, and handling methods have been extensively described in literature supplied by manufacturers (BASF Wyandotte Corp., 1972; Dow Chemical Company, 1977; Jefferson Chemical Company, undated a and b; Oxirane Corporation, undated) and safety information sources (NFPA, 1975; MCA, 1971). This literature chiefly concerns safety of humans and property. Tank cars for ethylene oxide and propylene oxide are specified as ICC-105A100W and 105A100. These are equipped with pressure relief valves which vent excessive pressure into the atmosphere. The epoxides should be stored in an area detached from the plant site and storage tanks should be diked. Ethylene oxide tanks should be equipped with cooling pipes. Tanks must be equipped with pressure relief valves, but specific instructions on emission control of excess pressure was not included. Vapor recompression systems could be applied to prevent emissions (Spencer, 1971).

5.3.3. Potential Environmental Formation. The major source of potential inadvertent production of ethylene oxide in the environment is probably the combustion of hydrocarbon fuels. Hughes et al. (1959) used gas-liquid partition chromatography to separate and identify oxygenated derivatives of hydrocarbons that were found in the combustion products of hydrocarbon fuels. Among the oxygenated combustion products identified were ethylene oxide and propylene oxide. Barnard and Lee (1972) also identified these compounds in the oxygenated products of n-pentane combustion. Seizinger and Dimitriades (1972) suggested that ethylene oxide is a component of automobile exhaust. They tested the combustion of simple unleaded hydrocarbon components of gasoline. Stationary sources of hydrocarbon combustion also might emit large quantities of these compounds into the environment.

Ethylene oxide has been identified in tobacco smoke (Binder and Lindner, 1972; Binder, 1974). It is not uncommon for tobacco to be treated with ethylene oxide by cigarette manufacturers for its fumigant properties. Binder and Lindner (1972) determined that the ethylene oxide concentration of unfumigated tobacco smoke was 0.02 μg/mℓ, while fumigated tobacco smoke had a concentration of 0.05 μg/mℓ and extensively fumigated tobacco smoke had a concentration of 0.30 μg/mℓ. Binder (1974) determined that the ethylene oxide content of smoke from unfumigated tobacco was 1 μg/g.

Epoxides are formed in the photochemical smog cycle. Olefins can be converted to the corresponding epoxides by reaction with an organic peroxide (Altshuller and Bufalini, 1965). Alkyl peroxides can decompose to yield an epoxide and oxy radical (NAS, 1976).

Water disinfection procedures might convert olefins to epoxides. Olefin conversion during chlorination of potable water would proceed by the same route as for chlorohydrination production of the epoxide (see Section 3.6.5). However, this process would require the conversion of ethylene to the chlorohydrin (Morris, 1975; Carlson and Caple, 1977). Since ethylene is very volatile, it seems unlikely that ethylene remains in water long enough for this process to occur significantly.

5.4. SUMMARY

This section discusses production, uses, and emission of ethylene oxide. Ethylene oxide is produced almost exclusively by direct oxidation of ethylene using either air or oxygen. Its 1981 production volume was 4937 million pounds, down from 5220 million pounds in 1980.

The major emission sources from production facilities are the main process vent for both air and oxygen units and the purge gas vent for air units; fugitive emissions are also a source of ethylene oxide in the atmosphere, although no emission estimate is available for this source. Total air emissions from production have been estimated to be around 2 million pounds based on 1978 production volume. Ethylene oxide also enters the atmosphere from handling, storage, and transfer operations, as well as the disposal of process wastes. There is no solid waste from ethylene oxide manufacture.

More than 90% of the ethylene oxide produced is used captively as a chemical intermediate, where there is some potential for environmental contamination. Up to 10 million pounds are used annually for fumigation and sterilization; ethylene oxide emissions from such uses might be significant. Ethylene oxide may also be produced by hydrocarbon combustion (e.g., automobile exhaust).

6. ENVIRONMENTAL FATE, TRANSPORT, AND DISTRIBUTION

6.1. INTRODUCTION

Epoxides are not persistent in the environment. Available information on their chemical and biological properties characterizes them as highly reactive. The available information was not sufficient to develop a definite description of their environmental transport characteristics. Interphase transport from water to air seems to be a slow process, but evaporation of ethylene oxide applied as a sterilant or a fumigant appears to be a rapid process. High water solubility and high vapor pressure result in significant mobility within water or air.

Epoxide degradation has been fairly well characterized, and indicates that ethylene oxide is reactive in all media. Available information on its ionic reactions indicates that chemical (see Section 3) and biological degradation produce the same degradation products. Its degradation in water, soil, commodities, and manufactured products proceeds through ionic reactions. Degradation in the atmosphere has not been well-characterized with respect to processes or products. Available information indicates that it is very reactive in photochemical smog cycle reactions. No information was available on whether ionic reactions (e.g., with water vapor or water within aerosols) significantly contributes to its degradation in the atmosphere.

6.2. ETHYLENE OXIDE FATE IN WATER

Ethylene oxide degrades in water by hydrolysis and related nucleophilic reactions; aqueous radical reactions are not a significant process. The hydrolysis chemistry of ethylene oxide has been discussed in Section 3.6.5, and the information presented there will be used in the present discussion.

Ethylene oxide has a hydrolysis half-life of 12.2 days in pure water, 12.9 days in filtered (0.22 μm filtered) Kanawha River water, and 14.2 days in unfiltered Kanawha River water (Conway et al., 1983). The Kanawha River water had a pH of 7.4 and the initial ethylene oxide concentration was ≃70 mg/ℓ. These variations in hydrolysis rates are well within the error limits of hydrolysis experiments discussed by Mabey and Mill (1978).

It is interesting to note that the presumed presence of a microbial population in the unfiltered river water did not decrease the half-life of the ethylene oxide. Although the microbial concentration was not reported, the lack of a significant change in degradation rate may indicate that biological reactions are not significant in river water. Also, it should be noted that a half-life of 12-14 days allows for exposure of biota and possibly humans to ethylene oxide, although the addition of hypochlorite in water treatment plants reduces the likelihood of human exposure. Conway et al. (1983) also reported that pH variations would have less of an effect on the rate of hydrolysis than temperature over a pH range of 5-10.

Evaporation from water also appears to be a significant removal process. Conway et al. (1983) reported the calculated relative desorption coefficient α_d ($\alpha_d = K_d$ (ethylene oxide)$/K_d(O_2)$, K_d is the desorption coefficient) to be 0.31, 0.34, and 0.36 for 10, 20, and 30° C water. Experimental values for 22° C water of 0.36 for no wind and 0.39 for a 5 m/s wind are reasonably consistent with a calculated value of 0.34 and may be the result of increased turbulence and wind flow. These values of α_d indicate that ethylene oxide will be desorbed from a body of water with a rate dependent upon the actual oxygen-transfer rate in a specific system. The rate of desorption will be less than that for volatile low solubility organic compounds such as toluene, benzene, and chloroform, which have an α_d of ≈0.65 (Rathburn and Tai, 1981).

Conway et al. (1983) also measured the BOD using 2 mℓ of domestic sewage/BOD bottle. They found biooxidation was 5, 22, 40, and 52% (of theoretical) on days 5, 10, 15, and 20, respectively. They suggested that in a sewage treatment plant, where the microbial population is much higher, biodegradation might be very fast; however, from their data it is not possible to determine whether the chemical degraded is actually ethylene oxide or whether it is ethylene glycol (from hydrolysis), since the hydrolysis half-life (≈14 days) is similar to the BOD half-life of slightly less than 20 days.

Hendry et al. (1974) reported the rate constant for the reaction of one epoxide with alkyloxy radical proceeding by α-hydrogen abstraction to be 8.5×10^4 M^{-1} s^{-1}/α-hydrogen, or 3.4×10^5 M^{-1} s^{-1} for ethylene oxide. Given an alkyloxy radical concentration in ambient water of 10^{-14} M, the half-life for this process is ≈6 years. Hence, hydrolysis and evaporation appear to be the dominant fate processes for ethylene oxide, while no definitive statement can be made regarding its biodegradation.

6.3. ETHYLENE OXIDE FATE IN SOIL

Pertinent data regarding chemical degradation of ethylene oxide in soil were not located in the available literature. It seems reasonable, given the composition of soil, that the half-life of ethylene oxide would be shorter in soil than in water.

6.4. ETHYLENE OXIDE FATE IN THE ATMOSPHERE

Little direct information on epoxide behavior in the atmosphere was available; however, some characteristics of ethylene oxide behavior can be inferred from the data on free-radical chemistry.

The atmospheric reactivity of volatile organic chemicals has been characterized by their relative reaction rates with hydroxyl radicals in the gas phase (Cupitt, 1980; Darnall et al., 1976); however, there are a number of difficulties in determining an atmospheric half-life or lifetime for ethylene oxide based on hydroxyl radical reactions. One important difficulty is in choosing the appropriate hydroxyl radical concentration: a number of different modeling and direct measurement efforts have provided a wide range of values for both average and altitude specific concentrations of hydroxyl radicals. A reasonable compromise for an average OH concentration is 1×10^6 molecules cm^{-3} based on more recent modeling efforts (Cupitt, 1983). For ground level, the concentration may be somewhat higher, possibly around 1.3 to 1.4×10^6 molecules cm^{-3} during the summer (Crutzen and Fishman, 1977; Logan et al., 1981). Using these two values, a temperature of 300 K, and the Arrhenius equation of Fritz et al. (1982) (see Section 3.6.5), the lifetime of ethylene oxide is found to lie somewhere between 100 days (using the upper limit of the Arrhenius equation) and 215 days (the lower limit) for a hydroxyl radical concentration of 1×10^6 molecules cm^{-3}, and between 74 days and 159 days (using the upper limit) for a hydroxyl radical concentration of 1.35×10^6 molecules cm^{-3}. This lifetime is in sharp contrast with the significantly shorter values found for other ethers. For example, tetrahydrofuran, a five membered cyclic ether, has a lifetime of ≃1 day. Fritz et al. (1982) suggested that this disparity is due to the distorted sp^3 bonds in ethylene oxide that give rise to a hydrogen-abstraction activation energy of 5.8 kcal/mol, higher than the standard 2.8 kcal/mol.

Bogan and Hand (1978) found the absolute rate constant of the reaction of oxygen atoms [$O(^3P)$] for ethylene oxide to be (6.3 ± 0.18) x 10^{-16} cm^3/mcule-sec at 300 K. This rate is several orders of magnitude slower than that for the hydroxyl radical reaction, and yields a half-life of 1400 years, given an atmospheric $O(^3P)$ concentration of 2.5 x 10^4 molecules/cm^3 (Graedel, 1978).

Sickles et al. (1980) measured the rate of ozone production in a Teflon^R smog chamber to rank 19 compounds relative to propane. The chambers were irradiated with sunlight outdoors. Purified air, the organic compound to be tested and NO_2 were added before sunrise to multiple chambers; ethylene oxide-to-NO_2 ratio at the onset of the experiment was 4:0.067. Ethylene oxide was much less reactive than propane. The rank order of reactivities found was: Acrylonitrile >perchloroethylene >ethanol >ethylacetate >acetone >methanol >acetic acid >propane >ethylene dichloride >acetylene >chloroform >dimethyl formamide >benzaldehyde >methylene chloride >pyridine >*ethylene oxide >methyl chloroform >phenol >acetonitrile >nitrobenzene. The relative ordering of compounds was similar in an indoor smog chamber study (Dimitriades and Joshi, 1977). The smog chamber study of Joshi et al. (1982) also concluded the low reactivity of ethylene oxide (half-life >53 hours). All of these results indicate that ethylene oxide is relatively unreactive in the atmosphere compared to other ethers.

With the information currently available, no definitive statement can be made regarding the atmospheric fate or lifetime of ethylene oxide.

6.5. DEGRADATION IN COMMODITIES AND MANUFACTURED PRODUCTS

Ethylene oxide is registered in the United States for use as a fumigant or sterilant for several stored-food commodities and manufactured products (Goncarlovs, 1983). These include its use as a fumigant for bulk food containers, other food containers, stored grain, stored fruits, stored processed foods, tobacco products, garments, furs, stored herbs and spices, furniture, aircraft, buses, railroad cars, and laboratory animal bedding. As a sterilant, it is used principally on hospital equipment and pharmaceuticals. Ethylene oxide is used as a fumigant chiefly to protect stored products from insect or microbial destruction. The fate of this epoxide and its residue are especially important in materials, commodities, and products coming into close contact with humans, such as surgical equipment, pharmaceuticals, and food service and packaging materials (Wesley et al., 1965; Alguire, 1973; Holmgren et al., 1969; Gilmour, 1978).

The study of the fate of ethylene oxide in these materials has established that it will degrade to glycol and halohydrin or evaporate. The degradation results from chemical and/or enzymatic activity. The halohydrin route requires epoxide reaction with inorganic halide. The halide could be naturally present, added or derived from organic halides. Bromide ion is often supplied by degraded methyl bromide, another fumigant (Rowlands, 1971; Lindgren et al., 1968).

Scudamore and Heuser (1971) measured the apparent degradation and evaporation of ethylene oxide and its residues, ethylene chlorohydrin and ethylene bromohydrin, over a 1-year period. Apparent first-order specific rate constants, k, were calculated for epoxide dissipation. The rate constant, k, combined losses from the degradation (chemical and metabolic pathways), k_D, and evaporation, k_V:

$$k = k_D + k_V$$

The glycols (ethylene and diethylene) were measured once at 6 months or 1 year after treatment. The parameters considered included the ethylene oxide treatment (dose and temperature during application), the moisture content of the commodity, storage temperature and type of storage (closed containers versus open trays). Ethylene oxide residues dissipated rapidly. While the estimated half-life was longest at 10°C in sealed containers, it never exceeded 2 weeks. Increasing the ethylene oxide dose did not have a simple effect on its loss rate. For the most part, small increases in the dose slightly decreased the loss rate, while very large increases caused larger decreases in the rate of loss and, sometimes, non-linear changes. The effect of moisture content appeared varied and relatively small. Scudamore and Heuser (1971) also monitored some commercially treated products and found ethylene halohydrin residues but no ethylene oxide residues. They concluded that ethylene oxide will usually dissipate from treated commodities, but, under some circumstances, small quantities could persist for several months.

Stijve et al. (1976) discussed the fate of ethylene oxide applied as a fumigant to commodities. They suggested that ethylene oxide could be retained by physical adsorption, but that it would persist not more than a few weeks before volatilization or reaction with natural constituents of the commodity.

Ben-Yehoshua et al. (1971) examined ethylene oxide residues during the treatment of dates. They reported a small ethylene oxide loss in the empty container and ascribed this to apparent adsorption to the container walls. The larger losses found with 2.1 kg of dates in the container were due to ethylene oxide uptake by the fruit. Ethylene oxide loss in treated dates left in open containers was attributed to degradation (to the chlorohydrin and glycol) and volatilization.

The available information on the fate of ethylene oxide applied to manufactured goods was less extensive as that on its fate in commodities. All available information suggests behavior similar to that discovered in commodities. Alguire (1973) described losses of ethylene oxide from polystyrene creamer cups and cream cheese wrappers at ambient temperature and open to the environment. The ethylene oxide did not degrade on the polystyrene cups, and was lost solely through out-gassing. More than 90% evaporated by the first day, and no residual ethylene oxide remained after 5 days. Ethylene oxide loss from cream cheese wrappers consisted primarily of conversion to ethylene glycol; no ethylene chlorohydrin was detected at any time. Ethylene oxide was completely gone by the tenth day.

Some studies have identified ethylene chlorohydrin residues in manufactured goods sterilized with ethylene oxide. These studies did not seek any information on volatilization losses. Brown (1970) identified ethylene oxide and its derivatives on treated equipment made of rubber, Dacron, and polyvinylchloride, but did not detect chlorohydrin on polyethylene equipment. Holmgren et al. (1969) measured 0-1500 ppm chlorohydrin on 21 ethylene oxide-treated drugs.

6.6. BIOACCUMULATION IN AQUATIC ORGANISMS

Specific experimental information regarding the bioaccumulation of ethylene oxide in aquatic organisms is not available. Veith et al. (1979) have suggested the calculation of BCF from the following equation:

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

where K_{ow} is the partition coefficient between octanol and water. Using this equation and the log K_{ow} of -0.30, reported by Hansch and Leo (1979), the BCF for whole fish was calculated to be 0.34.

6.7. SUMMARY

This section discusses the results of studies related to the fate of ethylene oxide in the environment. In water, ethylene oxide will degrade by hydrolysis and related nucleophilic reactions with a half-life of ≃12-14 days at 298 K. Lower temperatures lengthen the half-life; pH changes have minimal effects. Volatilization will also be a significant process although less so than for sparingly soluble compounds like toluene, chloroform or benzene. There is no conclusive evidence that microbial degradation is significant; however, the biological components of sewage sludge might react rapidly with ethylene oxide. The fate of ethylene oxide in soil will probably be similar to that in water; its half-life will probably be shorter.

The fate of ethylene oxide in the atmosphere is not obvious from the information present in the literature. Rate constants are available for hydroxyl radical and oxygen atom [$O(^3P)$] reactions as well as smog chamber studies. All predict that ethylene oxide will persist in the atmosphere, but the actual lifetime cannot be predicted.

In commodities, food containers, and manufactured goods, ethylene oxide appears to volatilize or to hydrolyze to glycol or halohydrin with a half-life of ≈2 weeks.

7. ENVIRONMENTAL LEVELS AND EXPOSURE

7.1. INTRODUCTION

The purpose of this document is to present available information relevant to human health effects that could be caused by this substance.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

7.2. ENVIRONMENTAL LEVELS

Ambient monitoring has portrayed ethylene oxide as an almost non-existent contaminant of environmental or biological samples. Although ethylene oxide is rarely identified in monitoring studies, its principal degradation products (glycols and halohydrins) have been found.

No monitoring data were available for ethylene oxide in biological tissues, except for some tissue-distribution studies. Since epoxides are reactive alkylating agents, it is reasonable to expect that ring-opening reactions will occur rapidly in biological systems so that the finding of detectable levels in environmental biota is unlikely (Anderson, 1971).

Only one ambient air monitoring study reporting the presence of ethylene oxide in air was found in the available literature. Bertsch et al. (1974) tentatively identified ethylene oxide in the ambient air near the University of Houston. However, the authors used Tenax as the adsorbant for trapping air contaminants and its use casts doubt on their tentative identification, since Tenax does not adequately retain ethylene oxide (see Section 4.1).

U.S. EPA (1976) listed one monitoring observation of ethylene oxide in water. It was observed in the effluent from a chemical plant in Brandenburg, Kentucky. No other epoxide observation was reported. U.S. EPA (1976) also noted observations of ethylene halohydrin, which might have been released in industrial wastes as such, rather than occurring as residues from epoxide.

No other reports of ethylene oxide in ambient air or water were found, yet Systems Application, Inc. (1982) reported that the maximum possible exposure concentration level of ethylene oxide, based on dispersion models, was 5 $\mu g/m^3$ (2.77 ppb). The justification for this value could be the reactivity of ethylene oxide or the lack of an adequate sampling method (see Section 4). Most sampling methods either lose significant amounts of ethylene oxide on even short-term storage, or use adsorbants with a very poor affinity for ethylene oxide (e.g., Tenax GC); however, this is certainly not the case for all studies, especially those using freeze-out techniques. Since environmental sample concentrations are rarely as high as workplace sample concentrations (particularly in the case of a reactive molecule such as ethylene oxide), the well documented NIOSH (1977) method becomes inadequate. The problem is compounded by the fact that few monitoring studies are undertaken to identify only a single compound in the environment. These studies must assume some compromise between completeness and speed, making it impossible to optimize conditions for the detection of any one compound.

Several studies have examined the residues of ethylene oxide that has been applied to commodities and manufactured goods as a fumigant and disinfectant. Another portion of this report (Section 6.5) describes investigations on the fate of this epoxide. The information here concerns residues in actual commercial products.

Scudamore and Heuser (1971) evaluated ethylene oxide and its metabolites in commercially treated products. While they never detected ethylene oxide in commercial products, they did find ethylene chlorohydrin residues ranging from 10-70 ppm. Lindgren et al. (1968) reviewed studies on residues from ethylene oxide treatment, most of which were fate studies rather than ambient monitoring studies. They suggested that residual epoxide could be present in commercial products.

Ethylene oxide is a common sterilant for surgical equipment. Its fate in plastic and rubber surgical equipment parallels its behavior in commodities. Brown (1970) monitored residues on various hospital equipment sterilized with ethylene oxide. Ethylene oxide was observed in three samples, one of which had received treatment ≈80 days previously. Ethylene chlorohydrin was detected in 10 samples.

7.3. EXPOSURE

The available data concerning the environmental levels of ethylene oxide are insufficient to properly estimate exposure; however, a general overview can be made. Over 5 billion pounds (>2 billion kg) of ethylene oxide is produced yearly. The vast majority is used captively as a synthetic intermediate. Perhaps 10 million pounds (4.5 million kg) is used for fumigation/sterilization for products that include food commodities, medical devices, pharmaceuticals, and cosmetics. This use constitutes the only documented potential exposure to ethylene oxide, though the extent of this exposure must be determined. Ethylene oxide also appears to be a product of incomplete combustion, and has been identified in automobile and diesel exhaust and in tobacco smoke. It can be formed during the photochemical smog cycle, but appears to be rapidly destroyed.

7.4. SUMMARY

This section discusses the results of monitoring studies conducted to measure the levels of pollutants, including ethylene oxide, in the environment. Very little information is available on ambient monitoring, no confirmed detection of ethylene oxide in air has been reported, and only one report exists for water. The lack of more monitoring reports may be because most, but not all, sampling methods would not detect ethylene oxide even if it were present. Several studies have examined the persistence and fate of ethylene oxide in commodities and commercial goods including food, medical supplies, and drugs.

8. ECOLOGICAL EFFECTS

8.1. MICROORGANISMS AND INSECTS

Ethylene oxide is used as a fumigant for foods (particularly grains) and spices, and shows major microbial, insecticidal, and acaricidal activity (Sykes, 1964; Lindgren and Vincent, 1966).

Fumigation with ethylene oxide has been used to control a wide variety of bacteria, fungi, rickettsiae and viruses. Sykes (1964), for example, reported that exposures to gaseous ethylene oxide at concentrations of 1-10% will kill *Bacillus globigii*, *Staphylococcus aureus*, *Escherichia coli*, *Chromobacterium prodigiosum*, and *Mycobacterium phlei* within a few hours. Roberts et al. (1943) found that 10% gaseous ethylene oxide will kill *Bacillus anthracoides* in 8 hours. Ethylene oxide also has significant sporicidal activity against dry bacterial spores (Bruch and Koesterer, 1961). Exposure of *Bacillus subtilis* spores to 1-2% vapor concentrations of ethylene oxide killed ≥95% of the spores within 4 hours. A 5% gaseous concentration of ethylene oxide produced 90% kill of airborne *B. globigii* spores in <2 hours (Roberts et al., 1943). Treatment of agar slants containing yeasts and fungi with 8% gaseous ethylene oxide for 3 hours was lethal to these microorganisms (Whelton et al., 1946). Skeehan (1959) indicated that herpes simplex, vaccinia, and bovine respiratory viruses are susceptible to saturated ethylene oxide vapor treatment.

Susceptible insects common to stored products include the flour beetle, rice weevil, and grain weevil (Lindgren et al., 1954). Ethylene oxide will kill one-half of the stored-product insect population at a concentration range of 6-18 mg/ℓ (Ong, 1948). Lindgren and Vincent (1966) reported a major reduction in available tissue glutathione content of Calliphora larvae exposed to ethylene oxide. Decrease in tissue glutathione via depletion of reduced-SH groups may be the mechanism of toxicity. The insect toxicity of ethylene oxide has been ranked by these authors as intermediate between those of ethylene dibromide and ethylene dichloride. A bibliography of ethylene oxide insecticidal properties, citing 185 references, has been published (Young and Busbey, 1935).

8.2. PLANTS

Pertinent data regarding the effects of exposure to ambient levels of ethylene oxide on plants were not found in the available literature. As detailed in Section 9.4, ethylene oxide is capable of inducing mutations and chromosomal aberrations in plants.

8.3. AQUATIC ORGANISMS

Limited information is available on the toxicity of ethylene oxide to aquatic organisms. The acute toxicity of ethylene oxide appears to be moderate, as indicated by LC_{50}s in the range of 84-90 mg/ℓ for fish, a mean 48-hour LC_{50} of 212 mg/ℓ for Daphnia and 745 mg/ℓ for brine shrimp (Table 8-1). LC_{50} values for the hydrolysis product ethylene glycol were >10,000 mg/ℓ for the above species except goldfish (which were not tested) (Conway et al., 1983). If reacted to form ethylene chlorohydrin, the 96-hour LC_{50} for fathead minnows was ≈90 mg/ℓ (Conway et al., 1983).

TABLE 8-1

Acute Aquatic Toxicity of Ethylene Oxide[a]

Test Procedure	Test Organism	LC_{50} (95% Confidence limits), mg/ℓ 24 hr	48 hr	96 hr	Reference
range-finding[b], static, aerated	fathead minnow	274 (150-500)	NA	NA	Conway et al., 1983
range-finding[b], static, sealed under oxygen	fathead minnow	86 (50-150)	NA	NA	Conway et al., 1983
definitive static acute[c] (no aeration)	fathead minnow	90 (63-125)	89 (63-125)	84 (73-96)	Conway et al., 1983
static acute	goldfish	90	NA	NA	Bridie et al., 1979
static acute	*Daphnia magna*	>300 270 260	300 137 (83-179) 200 (150-243)	NA NA NA	Conway et al., 1983
static acute	brine shrimp	>500 350 570	>500 1000 490	NA NA NA	Conway et al., 1983

[a] Source: Conway et al., 1983

[b] Range-finding tests used 2 fish/test concentration

[c] Definitive tests used 10 fish/test concentration

NA = Not applicable

9. BIOLOGICAL EFFECTS IN ANIMALS AND MAN

9.1. PHARMACOKINETICS

9.1.1. Absorption. Only limited data regarding the absorption of ethylene oxide were found in the available literature. However, acute toxicity data suggest that absorption occurs readily via the respiratory and gastrointestinal tracts (Table 9-1).

9.1.2. Distribution. Information concerning the distribution of ethylene oxide in the body is limited. Two studies have shown that it is found in many tissues following inhalation exposure or intravenous administration.

Ehrenberg et al. (1974) conducted inhalation studies with radioactively-labeled [1,2-^{3}H] ethylene oxide. Following exposure of mice to 1.15 ppm of the labeled chemical in air for 75 minutes, the highest levels of radioactivity (in unidentified chemical form) were associated with proteins isolated from the lungs, kidneys, and liver. Lower levels of radioactivity were measured in the testes, brain, and spleen, but additional organs were not analyzed.

Appelgren et al. (1977) carried out whole-body autoradiography on mice injected intravenously with radioactive [^{14}C]-ethylene oxide (label position unspecified). Preliminary inhalation studies with labeled ethylene oxide showed a similar tissue distribution of the compound similar to that seen following intravenous injection, except for a high initial labeling of the respiratory mucosa (data not shown). Two minutes after the injections, concentrations of radioactivity 2-3 times those seen in the blood were observed

TABLE 9-1

Acute Toxicity of Ethylene Oxide

Route	Species	Sex	Strain	Dose	Response	Reference
oral	rat	M	Wistar	330 mg/kg	LD_{50}	Smyth et al., 1941
oral	rat	M	NR	100 mg/kg	0/5 died	Hollingsworth et al., 1956
oral	rat	M	NR	200 mg/kg	5/5 died	Hollingsworth et al., 1956
oral	guinea pig	M,F	NR	270 mg/kg	LD_{50}	Smyth et al., 1941
oral	rabbit	M,F	NR	631 mg/kg	LD_{50}	Woodward and Woodward, 1971
ihl.	rat	M	white	1460 ppm/4 hours	LC_{50}	Jacobson et al., 1956
ihl.	rat	M,F	Sherman	4000 ppm/4 hours	LC_{50}	Carpenter et al., 1949
ihl.	guinea pig	NR	NR	7000 ppm/2.5 hours	LC_{low}	Waite et al., 1930
ihl.	mouse	F	white	835 ppm/4 hours	LC_{50}	Jacobson et al., 1956
ihl.	dog	M	beagle	960 ppm/4 hours	LC_{50}	Jacobson et al., 1956
i.v.	rabbit	M,F	NR	178 mg/kg	LD_{50}	Woodward and Woodward, 1971
i.v.	rat	M	NR	355 mg/kg	LD_{50}	Bruch, 1973
i.p.	rat	M,F	NR	178 mg/kg	LD_{50}	Bruch, 1973
i.p.	mouse	M,F	NR	178 mg/kg	LD_{50}	Bruch, 1973
i.p.	rabbit	M,F	NR	251 mg/kg	LD_{50}	Woodward and Woodward, 1971
s.c.	rabbit	M,F	NR	200 mg/kg	LD_{50}	Woodward and Woodward, 1971

Ihl. = inhalation; i.v. = intravenous; i.p. = intraperitoneal; s.c. = subcutaneous; NR = not reported in the liver, kidneys, and pancreas. Tissue labeling 20 minutes to 4 hours after exposure showed high levels of radioactivity in the liver, kidneys, lungs, intestinal mucosa, epididymis, cerebellum, and testes. Twenty-four hours after injection, radioactivity was still found in the liver, intestinal mucosa, epididymis, cerebellum, bronchi, and bone marrow. Since these observations were made on autoradiographs, quantitative results were not reported. The extent of bioexchange of the radioactive label into natural body constituents also could not be determined in this study.

9.1.3. Metabolism. Comprehensive studies designed to fully characterize the metabolic fate of ethylene oxide have not been conducted.

Significant concentrations of ethylene glycol were detected in the plasma of four beagle dogs following the intravenous administration of 25 or 75 mg/kg ethylene oxide on separate occasions (Martis et al., 1982). Urinary excretion data indicated that 7-24% of the administered dose was excreted in the urine within 24 hours as ethylene glycol; the mean percentages of the low and high doses that were excreted in the urine were 13.5 ± 3.5% and 14.2 ± 8.1%, respectively.

Two urinary metabolites were detected when [1,2-^{14}C] ethylene oxide was administered to Sprague-Dawley rats via a single intraperitoneal injection at a dosage of 2 mg/kg (Jones and Wells, 1981). The urinary metabolites were S-(2-hydroxyethyl)-cysteine (9% of the dose) and N-acetyl-S-(2-hydroxyethyl)-cysteine (33% of the dose), which suggests that the metabolism of ethylene oxide involved conjugation with glutathione. A small percentage of the dose was exhaled as $^{14}CO_2$ and as unchanged ethylene oxide (Section 9.1.4).

In the inhalation study with mice summarized in Section 9.1.2 (Ehrenberg et al., 1974), the only urinary metabolite characterized was 7-hydroxyethylguanine, which accounted for a minor amount (0.007%) of the total urinary radioactivity. Significant alkylation of tissue proteins was found, and alkylation of DNA was confirmed by the identification of a high specific activity radiolabeled 7-hydroxyethylguanine. Cumming et al. (1981) reported large differences in the patterns of initial alkylation as well as removal of total alkylation products from the DNA of various tissues (i.e., testis, liver, lung, kidney, spleen) of mice following inhalation exposure to tritium-labeled ethylene oxide. Thus, ethylene oxide distributes and reacts extensively throughout the body.

9.1.4. Elimination. In the inhalation study with mice (Ehrenberg et al., 1974) using tritium-labeled [1,2-^{3}H]-ethylene oxide (see Section 9.1.3), it was found that 78% (mean value) of the absorbed radioactivity was excreted in the urine within 48 hours. The biological half-life in mice was reported to be ≃9 minutes, indicating rapid urinary elimination.

Approximately 43% of the administered radioactive dose of [1,2-^{14}C] ethylene oxide (2 mg/kg, single injection) was excreted in the urine of mice over 50 hours, most of which (≃40%) appeared within 18 hours of dosing (Jones and Wells, 1981). Two urinary metabolites, S-(2-hydroxyethyl)-cysteine and N-acetyl-S-(2-hydroxyethyl)-cysteine accounted for 9 and 33% of the dose, respectively. Within 6 hours, 1.5% of the dose was exhaled as $^{14}CO_2$ and 1% as unchanged ethylene oxide, but these are not maximum values (exhaled radioactivity was not sampled at later post-exposure times).

Martis et al. (1982) investigated the elimination kinetics of intravenously administered ethylene oxide in beagle dogs. Four dogs received single 25 and 75 mg/kg injections of the compound on separate occasions, and venous blood was sampled for ethylene oxide and ethylene glycol at 0, 0.08, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 7.0, and 24 hours after administration. It was found that the ethylene oxide cleared rapidly from the plasma, and that in all cases concentrations decreased to <2% of the zero-time value within 5 hours. The plasma concentration of ethylene oxide declined exponentially, and first-order rate constants of 0.025 ± 0.006 min^{-1} and 0.023 ± 0.010 min^{-1} for the low and high dosages, respectively, were calculated from the plasma concentration corresponded to plasma half-lives of 29.3 ± 5.7 min and 36.5 ± 18.5 min. It was noted that the lack of significant differences in kinetic parameters (i.e., elimination rate constant, plasma half-life, apparent distribution volume, total body clearance) at the two dose levels indicates that the elimination kinetics are not dose-dependent. Ethylene glycol was formed quite rapidly following the administration of ethylene oxide, and plasma concentrations reportedly exhibited the characteristics of a metabolite in a one-compartment model; maximum plasma concentrations of ethylene glycol were reached by 90 ± 24.5 minutes (25 mg/kg) and 120 ± 42.4 minutes (75 mg/kg) post-injection. Plasma concentration-time data for ethylene glycol following the intravenous injection of 35 and 106 mg/kg of ethylene glycol indicated half-lives of 177.1 ± 29.3 and 264.9 ± 90 minutes, respectively.

9.2. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

9.2.1. Effects in Humans.

9.2.1.1. ACUTE EXPOSURE -- Case reports indicate that headache, nausea, vomiting, dyspnea, and/or respiratory irritation are common effects of acute inhalation exposure to ethylene oxide (Greaves-Walker and Greeson, 1932; Blackwood and Erskine, 1938; von Oettingen, 1939; Anonymous, 1947; Sexton and Henson, 1949; Hollingsworth et al., 1956; Curme and Johnston, 1952; Salinas et al., 1981). Symptoms of poisoning have been reported to be delayed by several hours following exposure. Similar effects (e.g., marked nausea and profuse vomiting), as well as mild leukocytosis and blisters (discussed subsequently), developed in three chemical plant workers who were dermally drenched with 1% aqueous ethylene oxide solution (Sexton and Henson, 1949). Inhalation exposure to high concentrations of ethylene oxide for brief periods has been associated with bronchitis, pulmonary edema, and emphysema (Thiess, 1963), as well as convulsive movements (Salinas et al., 1981). In a controlled study of the effects of ethylene oxide on human volunteers, Greaves-Walker and Greeson (1932) observed that ethylene oxide at ≈2200 ppm was slightly irritating to four subjects. At a 5-fold higher concentration, the compound had a definite effect on nasal mucosa within ≈10 seconds.

Three chemical plant workers drenched with 1% aqueous ethylene oxide solution developed marked nausea and profuse vomiting several hours following exposure (Sexton and Henson, 1949). Large vesiculated blisters developed in the areas of exposed skin, and two workers who had complete blood counts taken showed a mild leukocytosis.

Cobis (1977) reported a very low incidence of health-related effects due to exposure to ethylene oxide in Veteran's Administration medical facilities. Ethylene oxide was used for sterilization purposes in 162 hospitals and 7 outpatient clinics over an average of 8.2 years. Only 12 employees were reported to have been involved in exposure incidents, and symptoms included watering eyes, nausea, and skin irritation. These cases are currently being followed to determine possible exposure sequelae. The average exposure concentration was not given, and it is presumed (although not stated) that the employees were exposed to ethylene oxide vapor.

The dermatological effects of ethylene oxide contact have been reviewed by Taylor (1977). Concentrated ethylene oxide evaporates rapidly from the skin and produces a freezing effect, resulting in burns ranging from first- through third-degree severity. Ethylene oxide gas retained in porous materials that have not been properly aired can produce skin irritation. Foot burns (Phillips and Kay, 1949) and hand burns (Royce and Moore, 1955), for example, have been observed in workers that wore ethylene oxide-sterilized rubber boots and rubber gloves, respectively. Biro et al. (1974) described a hospital incident in which 19 women were burned by surgical gowns and drapes that had been sterilized with ethylene oxide. Joyner (1964) found in a 2-year retrospective study of medical records that ethylene oxide plant workers had experienced exposure-related burns.

Sexton and Henson (1949) described the dermatological reactions that occurred in 6 men whose skin was directly exposed to a 1% water solution of ethylene oxide for periods ranging from 15 minutes to 3 hours. The men with the maximum exposures (2-3 hours) exhibited the most marked cutaneous effects (vesicular eruptions), but nausea and vomiting were the only systemic effects noted.

In a subsequent study, Sexton and Henson (1950) applied 1-100% solutions of ethylene oxide to the skin of 8 volunteer subjects for time intervals that ranged from 20 seconds to 95 minutes. The magnitude of skin injury appeared to be related to the duration of contact and the concentration. The most hazardous concentrations of ethylene oxide were in the 50% range, since the manifestation arbitrarily examined in this study (minimal second-degree burn demonstrated as an area of erythema with one or more superimposed vesicles) was produced in 45 seconds with this solution. The degree of skin injury was proportionately decreased at concentrations both greater and less than 50%. The lowest ethylene oxide concentration investigated (1%) produced a mild reaction (erythema) after 50 minutes of exposure. The milder skin reactions at concentrations >50% were attributed to the fact that the more concentrated solutions boil vigorously, thus preventing efficient skin penetration; the more dilute solutions lacked sufficient chemical to cause injury except after prolonged contact. Delayed skin sensitization developed in 3 of the 8 subjects.

Shupack et al. (1981) demonstrated that human skin reactions were directly related to total dose when exposures were to ethylene oxide that was retained in permeable materials. In tests with 12 unsensitized volunteers, it was found that patch materials that rapidly lose ethylene oxide (i.e., fabric or rubber) elicited few reactions, even at ethylene oxide levels as high as 3000-5000 ppm after 4-8 hours of contact. Patch materials that lost ethylene oxide slowly produced mild skin reactions (erythema plus edema) at material levels as low as 1700 ppm (PVC film) and 1000 ppm (PVC blocks) after similar durations of contact. Patches were removed from the subjects after 1, 2, 4, and 8 hours; it was found that most of the ethylene oxide diffused from the fabric and rubber patches within 1 hour and from the PVC film patches within 4 hours, but that the PVC block retained a substantial portion of ethylene oxide residue at 4 hours. In a subsequent experiment the same subjects (i.e., those previously exposed in the first experiment) were exposed to patch materials that retained ethylene oxide the longest (thick PVC blocks and petrolatum applied to Webril pads). It was found that the reactions were most widespread when the ethylene oxide levels in these materials were ≈1000 ppm; erythema appeared in 10 of the 12 PVC block subjects and 10 of the 12 petrolatum subjects after 4-8 hours of contact, and cleared within 3-4 days. Reactions were not elicited at nominal levels of 10 or 100 ppm ethylene oxide in PVC or petrolatum, although one subject who had developed sensitivity to 1000 ppm ethylene oxide in PVC block in the first experiment showed a mild delayed reaction to 100 ppm. Little or no reaction developed to patches that contained ethylene oxide by-products that were present in the original patches (i.e., ethylene glycol and ethylene chlorohydrin), indicating that ethylene oxide was the toxic agent.

Although incidental findings in the Sexton and Henson (1950) and Shupack et al. (1981) experimental studies described above suggest that ethylene oxide can cause skin sensitization, Thiess (1963) did not observe sensitization in ethylene oxide plant workers who were challenged with a single dermal application of 1% after an average of 10.4 years of occupational exposure. Anaphylactic reactions have been observed in patients using ethylene oxide sterilized plastic tubing for hemodialysis (Poothullil et al., 1975) or cardiac catheterization (Pessayre and Trevoux, 1978). These symptoms included uticaria, breathlessness, and hypotension. In a follow-up study on a patient apparently sensitized by contact with hemodialysis tubing, Dolovich and Bell (1978) illustrated that this patient showed a positive skin test response to ethylene oxide-serum albumin conjugate, and produced in vitro histamine release to this antigen. This response indicates that a specific IgE antibody to ethylene oxide had been induced in this patient.

Clinical reports of hemolysis following use of ethylene oxide sterilized plastic tubings have also been published (Hirose et al., 1963; Clarke et al., 1966). Ethylene oxide, rather than a chemical reaction product, is implicated, since this type of effect can be prevented by extensive aeration of ethylene oxide sterilized plastic devices.

Ethylene oxide vapors in high concentrations are irritating to the eyes, but ocular contact with liquid ethylene oxide can cause severe burns. A workman exposed to ethylene oxide in an unstated manner was reported to have suffered a corneal burn, but healing was observed within 48 hours following a corneal denudement procedure (McLaughlin, 1946). Thiess (1963) described two cases of accidental eye injury with ethylene oxide. A nurse was exposed to a direct blast of ethylene oxide from a sterilizer cartridge, and developed an epithelial keratitis of the cornea within 3 hours. Within 24 hours, the eye was entirely normal. The second case involved a patient who received a squirt of liquid ethylene oxide (concentration not stated) in the eye and was treated immediately by extensive washing with water; this resulted in only irritation of the conjunctivae that persisted for ≈1 day.

9.2.1.2. SUBCHRONIC AND CHRONIC EXPOSURE -- Limited information is available on toxic effects of subchronic or chronic ethylene oxide exposure in humans. The information is largely derived from clinical case reports from retrospective mortality studies.

Gross et al. (1979) reported on four cases of apparent ethylene oxide-induced neurotoxicity. This occurred in a plant in which a sterilizer was found to have leaked for 2 months of operation. The exact levels of ethylene oxide were unknown, but the four individuals involved reported that they could intermittently smell the ethylene oxide gas, indicating roughly a level of >700 ppm. The length of exposure to ethylene oxide from the leaking sterilizer was 3 weeks for cases 1 and 2, 2 weeks for case 3, and 2 months for case 4. Three of the four cases had worked as sterilizer operators for >2 years and were exposed to ethylene oxide from the leaking sterilizer for 2, 3, or 8 weeks; the fourth had been an operator for only 3 weeks and was exposed for the duration.

The individual who had been exposed to ethylene oxide for 3 weeks had noted conjunctival and mucosal irritation and transient blunting of the senses of smell and taste, and developed headache, nausea, vomiting, and lethargy that was followed by acute encephalopathy (recurrent major motor seizures at 20-30 minute intervals). Two of the other three operators were symptomatic (i.e., headaches, numbness and weakness in the extremities, fatiguability, one case of memory/thinking disturbances) and had abnormal neurological examination results that were consistent with sensorimotor neuorpathy. Nerve conduction studies were abnormal in these three operators, including the asymptomatic patient, and were compatible with the diagnosis of sensorimotor neuropathy. Removal from exposure resulted in relief of symptoms within 2 weeks. Two of the individuals returned to work under normal conditions of lower ethylene oxide exposure, but improvement in nerve conduction was not observed;

significant improvement was noted, however, in the third individual who returned to work in a position without ethylene oxide exposure.

Jensen (1977) reported that three workers using ethylene oxide sterilizers were hospitalized for neuropathy of the lower limbs. Follow-up indicated that these effects were reversible.

Jay et al. (1982) found that the four sterilizer operators described above (Gross et al., 1979), who were exposed to excessive levels of ethylene oxide from a leaking sterilizer and developed neurologic abnormalities, subsequently developed cataracts. The operator exposed for 2 months developed bilateral cataracts during the following 2 1/2 years; cataracts were diagnosed in the other three operators upon examination ≈3 1/2 years after exposure to the leaking sterilizer. Eight other men whose work involved exposure to ethylene oxide sterilizers (6 of the 8 were sterilizer operators), but who were asymptomatic, were subjected to complete ocular examinations, but cataracts were not found. Four of the 12 men, two of whom had not worked on the leaking sterilizer, had increased central corneal thickness with normal endothelial cell counts when compared with a control group of 12 subjects of higher average age (41 vs. 33 years). None of the patients were examined before exposure to ethylene oxide, but the authors believed it unlikely that cataracts would occur by chance in persons in this age range, particularly because none of the patients had any systemic or ocular disease that might be associated with cataract formation.

Hemoglobin values and lymphocyte counts were reported to be significantly lower and higher, respectively, in a group of Swedish ethylene oxide production workers when compared with control subjects (Ehrenberg and Hallstrom, 1967). The design and results of this study are more completely described in

Section 9.5.2, but it should be noted that the production workers were reported to have been exposed for 2-20 years (average 15 years) to an unknown level of the compound.

Joyner (1964) conducted a retrospective morbidity study of 37 male ethylene oxide production plant workers. These workers varied in age from 29-56 years and were exposed to typical concentrations of 5-10 ppm (range 0-55 ppm) for 5-16 years (mean 10.7 years). Age-matched controls consisted of 41 operators (mean length of service, 11.7 years) assigned to other production units, who had past exposure to many different petrochemical industry agents, but had never exhibited clinical effects attributable to systemic chemical toxicity. As detailed in Sections 9.3 and 9.5, no significant increase in health problems relative to controls was found. This evaluation should have been sufficient to identify major toxic effects of extended low-level ethylene oxide exposure, although limitations in the design of the study, as well as an insufficient period of observation, preclude evaluation of more subtle toxic or carcinogenic responses.

An excess of deaths from specific causes (including all circulatory causes) other than certain malignancies (Section 9.5) was not observed in a group of 767 male ethylene oxide workers from the Texaco Chemical Company Plant in Port Neches, Texas (Morgan et al., 1981). These cohort members had been employed for at least 5 years between January 1955 and December 31, 1977, and an industrial hygiene survey of the plant performed in July, 1977, showed that the 8-hour TWA exposure to ethylene oxide was well below 50 ppm.

Hogstedt et al. (1979a) conducted a cohort study of mortality among 89 full-time ethylene oxide production workers, 86 intermittently exposed maintenance workers, and a group of 66 unexposed control workers during the years

1961-1977. As described in Section 9.5, exposure patterns were quite complex; in addition to ethylene oxide (concentrations were generally <50 mg/m^3), workers were exposed at different times to ethylene dichloride, ethylene chlorohydrin, ethylene, low concentrations of bis(2-chloroethyl)ether, as well as traces of other chemicals. It was found that the full-time exposed cohort showed considerable excess mortality when compared with the number expected based on national statistics. The excess mortality arises mainly from increased mortality due to stomach cancer and leukemia (Section 9.5), but also from diseases of the circulatory system. When at least 1 year of exposure and $\geq$10 years of induction-latency time were required for inclusion in the study, there were 12 observed deaths attributed to the circulatory system (9 due to coronary heart disease and 3 due to cerebrovascular disease), compared to the expected incidence of 6.3; this difference was statistically significant ($P<0.05$). The excess mortality was of the same magnitude in a restricted cohort of those with $\geq$10 years of employment in ethylene oxide production and 20 years of induction-latency time (7 observed, 2.2 expected).

9.2.2. Effects in Animals.

9.2.2.1. ACUTE EXPOSURE -- The acute toxicity of ethylene oxide is summarized in Table 9-1. Exposure of mice, rats, guinea pigs, rabbits, and dogs to lethal levels of ethylene oxide has produced symptoms of mucous membrane irritation and CNS depression, including lacrimation, nasal discharge, salivation, nausea, vomiting, diarrhea, respiratory irritation, incoordination, and convulsions (Sexton and Henson, 1949; Hollingsworth et al., 1956; Hine et al., 1981). Animals that survived the initial exposures showed subsequent bronchitis, pneumonia, and loss of appetite, with delayed symptoms of apathy, dyspnea, vomiting, paralysis (particularly of the hindquarters), periodic convulsions, and death (Waite et al., 1930; Hollingsworth et al., 1956). Prompt deaths are usually due to lung edema; delayed deaths frequently result from secondary infections in the lungs, although general systemic intoxication may also be a factor (Hine et al., 1981).

Pathological findings following lethal exposure to ethylene oxide in mice, rats, and guinea pigs showed congestion of the lungs, hyperemia of the liver and kidneys, and gray discoloration of the liver (Waite et al., 1930). Pathological findings after delayed death caused by ethylene oxide included emphysema of the lungs, fatty degeneration of the liver, cloudy swelling of the kidney tubules, and congestion of the spleen and brain (Hollingsworth et al., 1956). Intravenously-administered ethylene oxide caused congestion in all organs of the rabbit (Greaves-Walker and Greeson, 1932). Zamlauski and Cohen (1976) have reported that infusion of ethylene oxide in the rat at blood levels of 0.45-4.5 mg/mℓ produced a significant decrease (≈30%) in glomerular filtration rate, which indicates effects of ethylene oxide on kidney function.

Ethylene oxide in 10 and 50% aqueous solutions produced hyperemia and edema in shaved rabbit skin when applied through cotton pads for 1-60 minutes (Hollingsworth et al., 1956). Bruch (1973) studied the dermal irritation properties of 2-10% aqueous ethylene oxide solutions in guinea pigs and rabbits. Subcutaneous injection in the guinea pig resulted in ecchymoses and skin thickening, while intradermal injection and topical application in the rabbit resulted in mild irritation. Topical or intradermal administration of 1% ethylene oxide (0.5 mℓ), thrice weekly for 3 weeks, did not result in sensitization in guinea pigs (Woodward and Woodward, 1971).

McDonald et al. (1977) studied the ocular effects of varied concentrations of ethylene oxide in saline applied repeatedly over a 6-hour period to the eyes of rabbits. They observed a dose-dependent increase in congestion, swelling, discharge, iritis, and corneal cloudiness, indicating the irritating effect of ethylene oxide on mucous membranes and corneal epithelium. The maximum nondamaging concentration for this time period was 0.1% ethylene oxide. In another study of ocular irritation in rabbit eyes, Woodward and Woodward (1971) found slight irritation following a single application of 10% aqueous ethylene oxide (duration of exposure unknown), and a no-effect concentration of 2.1% ethylene oxide was determined. The higher values determined in this study are probably the results of a different mode of application and, therefore, different duration of exposure.

9.2.2.2. SUBCHRONIC AND CHRONIC EXPOSURE -- The subchronic toxicity of inhaled ethylene oxide has been investigated in a variety of different animal species by different routes of exposure (Hollingsworth et al., 1956; Jacobson et al., 1956). As summarized in Table 9-2, symptoms of poisoning and pathologic changes are similar to those observed in acute studies, including lung, kidney, and liver damage, and neuropathy of the hindquarter and testicular tubule degeneration in some species.

Hollingsworth et al. (1956) observed neurotoxic effects in animals following inhalation exposure to 357 ppm ethylene oxide vapor for several weeks (the exposure for each species is presented in Table 9-2). Rats, rabbits, and monkeys showed paralysis and atrophy of the muscles of the hind limbs. These effects were reversible 100-132 days after discontinuation of exposure. Special studies on monkeys were carried out with repeated (38-94)

TABLE 9-2

Subchronic Toxicity of Ethylene Oxide

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	20 rats (10/sex) 16 guinea pigs (8/sex) 5 mice (female) 2 rabbits (1/sex) 1 monkey (female)	841 ppm	up to 8 in 10 days (7 h/d; 5 d/wk)	Death in all animals. Pathologic changes in lungs, liver and kidneys similar to those in acute poisoning.	Hollingsworth et al., 1956
inhalation	30 mice (female, white) 20 rats (male, white)	400 ppm	30 (6 h/d; 5 d/wk)	Weight loss, reddish nasal discharge, diarrhea, labored breathing, weakness of the hind legs, and some deaths (13/20 exposed and 0/20 control rats, and 24/30 exposed and 3/30 control mice). Fifteen additional rats or mice were examined pathologically; changes were limited to a few cases of hemosiderosis in the spleen that occurred late in the exposure period.	Jacobson et al., 1956
inhalation	20 rats (10/sex) 10 mice (female)	357 ppm	33-38 (7 h/d; 5 d/wk)	Death in 10/10 mice (33 exposures) and 18/20 rats (38 exposures) caused by secondary respiratory infections. Impairment of sensory and motor function in rats prior to death, resulting in reversible hind leg muscle paralysis and atrophy.	Hollingsworth et al., 1956
inhalation	16 guinea pigs (8/sex)	357 ppm	123 in 176 days (7 h/d; 5 d/wk)	Growth depression, degeneration of the testicular tubules with replacement fibrosis (males), slight fatty degeneration of the adrenal cortex (females). No nervous system effects or mortality.	Hollingsworth et al., 1956

TABLE 9-2 (cont.)

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	2 monkeys (1/sex) 2 monkeys (males)	357 ppm 357 ppm	38-41 in 60 days 94 in 140 days (both schedules 7 h/d; 5 d/wk)	Growth depression and characteristic neurological impairment (e.g., hind limb paralysis and muscular atrophy, poor or nonexistent knee reflex, extensor reflex and hindquarter/genitalia pain perception). No histopathologic effects of exposure.	Hollingsworth et al.,1956
inhalation	3 dogs (male, Beagle)	290 ppm	30 (6 h/d; 5 d/wk)	Two of 3 exposed dogs showed toxic signs that included vomiting, slight tremors, transient weakness of the hind legs and decreases in red blood cells, hemoglobin, and hematocrit. Hematologic parameters normal in control dogs. Lungs showed congestion and alveolar collapse and fatty changes in the hindquarters were consistent with muscular atrophy.	Jacobson et al., 1956
inhalation	20 rats	204 ppm	127-133 in 185-193 days (7 h/d; 5 d/wk)	Weight loss, some deaths with effects on lungs (congestion, hemorrhage, emphysema, atelectasis) kidneys and testes (slight degeneration of some tubules) (slight cloudy swelling of tubules)	Hollingsworth et al., 1956
inhalation	60 mice (30/sex)	250 ppm	50-55 (6 h/d; 5 d/wk)	Signs of neuromuscular toxicity, decreased red blood cell count, packed cell volume and hemoglobin concentration were ob- in both sexes. No histopathologic effects were observed.	Snellings et al., 1984a
inhalation	8 guinea pig 4 rabbits (2/sex) 2 monkeys (female)	204 ppm	127-157 in 176-226 days (7 h/d; 5 d/wk)	No effect on growth or mortality. Evidence of paralysis/muscular atrophy in the rabbits and monkeys. Slight edema and congestion noted in rabbits' lungs.	Hollingsworth et al., 1956

TABLE 9-2 (cont.)

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	20 rats 8 guinea pigs 4 rabbits (2/sex) 2 monkeys (females)	113 ppm	122-157 in 176-226 days (7 h/d; 5 d/wk)	Growth depression and a moderate increase in lung weights in rats were the only adverse treatment-related effects noted.	Hollingsworth et al., 1956
inhalation	60 mice (30/sex)	100 ppm	50-55 (6 h/d, 5 d/wk)	Hunched posture and reduced locomotion observed in both sexes. No histopathologic effects were observed.	Snellings et al., 1984a
inhalation	30 mice (females, White) 20 rats (male, White)	100 ppm	130 (6 h/d, 5 d/wk)	No clinical signs of toxicity or treatment related mortality (3/20 exposed and 3/20 control rats, and 8/30 exposed and 4/30 control mice died). No significant pathologic changes in additional groups of 60 rats or mice.	Jacobson et al., 1956
inhalation	240 rats (120/sex)	100 ppm	145 (6 h/d, 5 d/wk)	Early deaths and decreased body weight gain were observed starting at week 4.	Snellings et al., 1984b
inhalation	3 dogs (male, Beagle)	100 ppm	130 (6 h/d, 5 d/wk)	Normochronic anemia (decreased red blood cell, hemoglobin, and hematocrit) indicated in 1 and suggested in 1 of 3 dogs. No changes in the 3rd exposed dog, or in control dogs.	Jacobson et al., 1956
inhalation	60 mice (30/sex)	50 ppm	50-55 (6 hr/d, 5 d/wk)	Hunched posture in males and reduced locomotion in females. No histopathologic effects were observed.	Snellings et al., 1984a
inhalation	20 rats 8 guinea pigs 4 rabbits (2/sex) 10 mice (female)	49 ppm	127-131 in 180-184 days (7 h/d, 5 d/wk)	No adverse effects as judged by general appearance, behavior, mortality, growth, final body and organ weights, and gross or microscopic pathologic examination.	Hollingsworth et al., 1956

TABLE 9-2 (cont.)

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	240 rats (120/sex)	33 ppm	145 (6 h/d, 5 d/wk)	Decrease in body weight gain starting at week 10.	Snellings et al., 1984b
inhalation	60 mice (30/sex)	10 ppm	50-55 (6 h/d, 5 d/wk)	No effects were observed.	Snellings et al., 1984a
inhalation	240 rats (120/sex)	10 ppm	145 (6 h/d, 5 d/wk)	No effects were observed.	Snellings et al., 1984b
oral (intubation)	5 rats (female)	100 mg/kg	15 doses in 21 days (5 d/wk)	Weight loss, gastric irritation and slight liver damage, but no mortality.	Hollingsworth et al., 1956
oral (intubation)	rats (female)	30, 10 or 3 mg/kg	22 doses in 30 days (5 d/wk)	No evidence of adverse effect as indicated by growth, hematology, blood urea nitrogen determinations, organ weights or gross microscopic pathology.	Hollingsworth et al., 1956
s.c.	rats	54 mg/kg	30	Weight loss, injection site hemorrhage and inflammation.	Hollingsworth et al., 1956
s.c.	rats	18 mg/kg	30	No observed effect.	Hollingsworth et al., 1956
s.c.	dogs	36 mg/kg	30	Anemia, hyperplastic bone marrow, and ectopic hematopoiesis.	Woodward and Woodward, 1971
i.v.	dogs	36 mg/kg	21	No observed anemia, other observations not mentioned.	Balazs, 1976

d = day; h = hour; wk = week

exposures to this level of ethylene oxide. Knee jerk reflexes became very weak, pain perception in the hind quarters decreased, the cremasteric reflex was elicited, and the extensor reflex of the palms of the hind feet was abolished. Impairment of both sensory and motor function at the lumbar and sacral level of the spinal cord was indicated. Exposure of monkeys to a lower level of ethylene oxide (204 ppm for 176-226 days) produced partial paralysis and some muscular atrophy of the hind legs with moderate suppression of the leg reflexes. The Babinski reflex was present after this lower level exposure to ethylene oxide.

In a more recent subchronic study, Snellings et al. (1984a) exposed groups of 30 male and 30 female B6C3F1 mice to the vapors of ethylene oxide. Exposures were for 6 hours/day, 5 days/week for 10-11 weeks to nominal levels of 1, 10, 50, 100, and 250 ppm. No effects were observed on survival, body weight or histologic sections of a variety of organs. At the three higher exposure levels, however, signs of neuromuscular toxicity were observed. In both sexes of the high exposure group, there was a statistically significant increase in hunched posture, reduced locomotion, and righting reflex. The former two were observed in the 100 ppm group and in males and females, respectively, of the 50 ppm group. The abnormal righting reflex was observed only during intermediate testing of females in the 100 ppm group. In addition, reduced toe pinch reflex was reported for females tested at intermediate periods and reduced tail pinch reflex was reported for males at termination in the 250 ppm group. Also at termination in the high dose group, hematologic parameters, red blood cell count, packed cell volume and hemoglobin concentrations were decreased, and some changes in either absolute or relative organ weights were observed. In this study, the neuromuscular effects appeared to be the most sensitive indicator of exposure to ethylene oxide.

Preliminary results of a chronic inhalation study conducted by NIOSH have been reported (Lynch et al., 1982a). Male F344 rats (80 per treatment group) and male Cynomolgus monkeys (12 per treatment group) were exposed to either 50 or 100 ppm ethylene oxide for 7 hours/day, 5 days/week for 24 months. Additional details of the experimental design are presented in Section 9.5, but it should be noted that the rats were included primarily for carcinogenicity evaluation, and the monkeys used to determine target organ toxicity. A number of indices were evaluated including body weights, hematology, clinical chemistry, urinalysis, opthalmology, pulmonary function, neurophysiology, neuropathology, gross and histopathology, sister chromatid exchange rates, and chromosomal aberrations in peripheral lymphocytes. The results that are currently available are summarized below.

As detailed in Section 9.5, weight gain throughout most of the exposure and survival periods were significantly depressed in the rats at both exposure levels (Lynch et al., 1982a). Weight gain was significantly depressed in the treated monkeys beginning at week 25. The liver and spleen of the rats were the only organs in which histopathological evaluations have been completed, but the preliminary terminal sacrifice spleen data indicate a dose-related induction of leukemia (Section 9.5). Hematologic analyses showed no statistically significant change in red blood cell count in the treated rats, but white blood cell counts were highly variable and reflected the presence of the leukemia. There were no differences in the red or white blood cell counts in either of the monkey groups, although increased frequencies of chromosomal aberration and SCE were observed in the peripheral lymphocytes of these animals.

In another 2-year inhalation toxicity study in rats conducted by Snellings et al. (1984b), the only non-neoplastic effect reported was a decrease in body weight gain. As described more fully in Section 9.5, groups of 120 male and 120 female Fischer 344 rats were exposed 6 hours/day, 5 days/week to ethylene oxide at target levels of 0, 10, 33, and 100 ppm. Decreased body weight gain was observed in both sexes after 4 weeks in the high exposure group and in females of the 33 ppm group after 10 weeks. Other groups were similar to control animals. Early deaths were reported for the high exposure group and were likely to be tumor-related. The incidence and type of neoplastic lesions are discussed in Section 9.5.

Significant hematological effects (i.e., anemia) have also been observed in ethylene oxide-exposed dogs. Jacobson et al. (1956) found decreased red blood cell counts, hemoglobin, and hematocrit in 2 of 3 beagle dogs that were exposed to 292 ppm ethylene oxide vapor for 6 hours/day, 5 days/week for 6 weeks. Definite (1 dog) and suggestive (1 dog) hematologic effects of the same type were also observed in 2 of 3 dogs that were similarly exposed to 100 ppm ethylene oxide for 6 months. Woodward and Woodward (1971) demonstrated a dose-related increase in anemia in dogs that were administered 6-36 mg/kg ethylene oxide in 30 daily subcutaneous injections. Pathologic examination showed hyperplastic bone marrow and ectopic hematopoiesis. Balazs (1976), however, was unable to repeat these findings in beagle dogs with an ethylene oxide-glucose solution administered intravenously over the same concentration range in a 21-day study.

An oral feeding study using 10% ethylene oxide in olive oil was performed on rats (Hollingsworth et al., 1956). Rats fed 100 mg/kg ethylene oxide in 15 doses over 21 days showed marked weight loss, gastric irritation, and slight liver damage. Feeding of 30 mg/kg in 22 doses produced no observable adverse effects.

9.2.3. Summary of Toxicity. The primary effects of acute inhalation exposure to high concentrations of ethylene oxide gas are respiratory tract irritation and CNS depression. Headache, vomiting, dyspnea, and diarrhea are common systemic effects of vapor exposures in humans, and excessive exposures have produced bronchitis, pulmonary edema, and convulsive movements. Similar effects have been observed in a variety of animal species, but paralysis (particularly of the hindquarters) and periodic convulsions frequently preceded death. Death in ethylene oxide-exposed laboratory animals is usually due to lung edema or secondary lung infections, and postmortem pathologic findings in other organs include widespread hyperemia and congestion (liver, kidneys, spleen) and fatty degeneration (liver).

Dermatological effects following skin contact with ethylene oxide in humans from accidental or experimental exposure include edema, erythema, and vesiculation with possible bleb formation, in that sequence. Vesicle formation is usually delayed (e.g., 6-12 hours), the magnitude of skin injury appears to be related to concentration and duration of contact, and the effects are reversible. Concentrated ethylene oxide evaporates from the skin resulting in a freezing effect, but more dilute solutions penetrate the skin more effectively, resulting in chemical burning; weak solutions lack sufficient chemical strength to cause injury except after prolonged contact. Skin burns have also been caused by residual ethylene oxide in clothing or footwear treated or accidentally contaminated with the compound. Sensitization has also been associated with repeated dermal exposure to ethylene oxide at the sites of contact. Similar dermal irritative effects have been observed in experimentally exposed rabbits and guinea pigs, but sensitization was not demonstrated by topical or intradermal administration in guinea pigs. High concentrations of ethylene oxide vapors are irritating to the eyes of humans and animals, and direct ocular contact with liquid ethylene oxide can produce corneal injury.

Case reports indicate that neurological effects (e.g., headache/vomiting, sensorimotor neuropathy, seizures) and ocular effects (e.g., cataracts) may be primary effects of limited repeated exposure to high levels of ethylene oxide, and hematological effects (reduced hemoglobin and increased number of lymphocytes) have been noted in chronically exposed ethylene oxide production plant workers. Retrospective morbidity and mortality studies of ethylene oxide production workers do not, however, suggest chemical-related, non-neoplastic toxicity. Subchronic exposure of different species of animals to ethylene oxide by different routes of exposure produced effects similar to those seen in acute studies; symptoms of poisoning primarily reflect neurotoxic action (e.g, hindquarter neuropathy) and pathologic changes generally occur in the lungs, kidney, and liver (e.g., congestion and degenerative changes), although testicular effects (e.g., tubule degeneration) and hematologic effects (e.g., anemia) have also been observed.

9.3. TERATOGENICITY AND REPRODUCTIVE TOXICITY

9.3.1. Teratogenic Effects. Batelle Pacific Northwest Laboratories (Hackett et al., 1982) conducted teratology and reproductive studies for the National Institute for Occupational Safety and Health investigating the effects of ethylene oxide produced by inhalation exposure. Pregnant rabbits and rats were exposed to a single dose of ethylene oxide (150 ppm, Union Carbide, Linda Lot No. 01901, 99.7% pure) both prior to and during the period of organogenesis. Thirty New Zealand White rabbits per group were exposed in three different regimes (filtered air alone, ethylene oxide exposure on days 7-19 of gestation, and ethylene oxide exposure on days 1-19 of gestation). Forty-one Sprague-Dawley CD rats per group were exposed according to four different schedules (filtered air alone, ethylene oxide exposure on days 7-16 of gestation, ethylene oxide exposure on days 1-16 of gestation, and ethylene oxide exposure 3 weeks prior to mating and through days 1-16 of gestation).

In the rabbits, no toxic effects were observed in the mothers (i.e., changes in body weight, organ weight, histopathological changes in the organs). In addition, there were no decreases in the percentage of pregnant animals nor was there any indication of adverse effect on the fetus (i.e., decreases in fetal body weight, crown rump length, sex ratios, or morphologic alterations).

In the rats, maternal toxicity was observed with sporadic decreases in food consumption, decreases in body weight, and increases in kidney and spleen weights, with the increases in spleen weights roughly proportional to the duration of exposure. Adverse effects were also observed in the developing conceptus. There was an increase in resorptions in animals exposed both pre-

and postgestationally with a trend for early midgestational resorptions. In addition, lowered fetal body weight, decreased crown-rump length and an increased incidence of incomplete skeletal ossification were observed in all ethylene oxide exposed offspring, and this was especially pronounced in animals exposed both pre- and postgestationally. It was concluded from this study that exposures of 150 ppm in rats caused significant adverse effects in both the mother and developing fetus; however, since only one dose was used in this study, it is not known whether these developmental effects would occur in the absence of maternal toxicity.

Because of concerns over adverse reproductive effects which could occur as a result of exposure to ethylene oxide or ethylene oxide reaction products left on improperly degassed surgical supplies, LaBorde and Kimmel (1980) conducted studies on the effects of ethylene oxide administered intravenously. CD-1 mice in four replicates of three treatment groups (10 animals per group) were treated with 0, 75, 150 mg/kg ethylene oxide (Eastman Organic Chemicals Co., purity not stated, ethylene oxide was injected in 5% dextrose solution). The animals were exposed in the following treatment periods of gestation: days 4-6 (period I), days 6-8 (period II), days 8-10 (period III) and days 10-12 (period IV).

Clinical signs of maternal toxicity (weakness, labored breathing, tremors, and death) were observed in animals injected with 150 mg/kg ethylene oxide on gestational days 4-6 (period I), 8-10 (period III) and 10-12 (period IV) but not in the group injected on days 6-8 (period II). Decreases in mean maternal body weight gain were observed in animals in period I, period III, and period IV and were accompanied by decreases in the mean number of live fetuses in periods III and IV. Embryotoxicity as manifested by significant reductions in mean fetal weight was observed in all four periods at the 150 mg/kg dose. There was no significant change in the mean number of implants per litter, but there was a reduction in the mean number of live fetuses per litter (and an increase in the number of dead and resorbed offspring) in periods III and IV at the 150 mg/kg level. An increase in the percent of malformed fetuses/litter was noted in periods II, III, and IV at 150 mg/kg level, but in period III the incidence did not achieve statistical significance. It was concluded that the ethylene oxide exposure under these conditions was selectively affecting the development of the conceptus (as seen by skeletal malformations and embryotoxicity) since ethylene oxide exposure in period II (days 6-8 of gestation) produced malformations and embryonic death while not affecting the mother (no clinical signs of toxicity); however, this conclusion was tempered somewhat by the observation of maternal deaths in treatment groups before and after this time period. Although there was no dose-response relationship in the severity of adverse effects in either the mother or fetus, the types of malformations seen in periods II and III appeared to follow a developmental pattern. The authors reported that in animals treated on days 6-8 cervical and upper thoracic vertebrae malformation were observed. Animals treated on days 8-10 had defects primarily in the lower thoracic region.

Another study by the same investigators (Jones-Price et al., 1983) evaluated the reproductive effects of intravenous injections of ethylene oxide in rabbits. New Zealand White rabbits were intravenously injected in two treatment regimes; 0, 9, 18, or 36 mg/kg ethylene oxide (source and purity not reported) on days 6-14 of gestation, or 0, 18, or 36 mg/kg on days 6-9 of gestation. Seventeen to 21 animals were examined in the group exposed on days 6-9, 18-24 animals examined in the group exposed on days 6-14.

Maternal toxicity was observed in both exposure groups, with more severe effects observed in the groups treated on days 6-14 than on days 6-9 of gestation. Significant decreases in maternal weight gains were observed during the entire treatment at the 18 and 36 mg/kg level. These decreases included both decreases in pregnancy weight gains and decreases in absolute weight gains (weight gained during pregnancy minus uterine weight). No embryotoxic effects were observed in the day 6-9 treatment groups; however, in the 6-14 day treatment group significant dose-related trends for decreased numbers of live fetuses/litter and resorptions/litter were observed. At the 36 mg/kg level, the incidence of resorptions/litter was statistically significantly different from control levels. The authors concluded that intravenous administration of ethylene oxide in rabbits produced embryotoxicity, though at doses which also produced significant maternal toxicity.

LaBorde et al. (1982) presented data at the 1982 Society of Toxicology meeting regarding the teratogenic effects of ethylene chlorhydrin (ECH), a reaction product of ethylene oxide, in mice and rabbits. ECH is produced by the interaction of ethylene oxide and chloride ions, so it is a residue of ethylene oxide that could be left on medical devices after improper degassing of ethylene oxide during sterilization. Forty-one to 65 CD-1 mice were injected intravenously with 60 mg/kg or 120 mg/kg ECH (source not reported; in 5% sterile dextrose) on days 4-6, 6-8, 8-10, or 10-12 of gestation. Seventeen to 22 New Zealand White rabbits were intravenously injected with 9, 18, or 36 mg/kg ECH on days 6-14 of gestation.

In this study, no adverse effects were observed in either the mother or the fetus of the New Zealand White rabbits; however, in CD-1 mice, clinical signs of toxicity (weight loss of $\geq$1 g in 24 hours) were observed in the mothers in all treatment periods at the 120 mg/kg dose. Maternal weight gain during the entire treatment period and during pregnancy were significantly reduced at the 120 mg/kg level in day 4-6, 6-8, and 10-12 groups. There was also a trend for increased resorptions/litter in animals exposed on days 4-6 and 10-12 at the 120 mg/kg level. At the 120 mg/kg dose for all treatment periods, there was a significant decrease in mean fetal weight/litter. At the 60 mg/kg level in animals exposed on days 8-10, there was a significant reduction in fetal weight in the absence of maternal toxicity. The authors reported a trend for an increase in the number of malformed fetuses treated on days 8-10; however, the incidence of this effect did not achieve statistical significance.

The conclusion reached by LaBorde et al. (1982) was that ECH administered intravenously in mice produced embryo/fetal toxicity and possibly a slight increase in malformations at maternally toxic doses. At the 60 mg/kg level, in animals treated on days 8-10, fetal weight reductions occurred in the absence of maternal toxicity; therefore, it was concluded that ECH may pose a hazard specific to the developing conceptus.

Verret (1974) investigated the toxic and teratogenic effects of ethylene chlorohydrin (ECH) in the developing chick embryo. ECH (source and purity not reported) was administered via the air cell during a pre-incubation period (0 hour) and after 96 hours of incubation at levels equivalent to 10, 25, 50, 100, and 200 mg/kg. The control groups were treated with the vehicle (water) or left untreated. One hundred eggs were used per group. Ethylene chlorohydrin was found to be toxic in this system with significantly increased mortality (no hatch) at levels ≥ 25 mg/kg at the 0 hour exposure, and at levels ≥ 12.5 mg/kg at the 96 hour exposure. Statistically significant increases in the number of structural anomalies were observed at two dose levels (50 and 100 mg/kg) at the 0 hour exposure, and at four dose levels (12.5, 25, 50, and 100 mg/kg) at the 96 hour exposure. The significance of these observations in terms of mammalian effects is not known, however, since teratogenic effects in chick embryos may not be predictive of mammalian effects.

9.3.2. Reproductive Effects. The Carnegie-Mellon Research Institute (Snellings et al., 1982a) conducted a one-generation study evaluating the reproductive effects of inhalation exposure to ethylene oxide. Thirty male and female Fischer-344 rats were continually exposed to 10, 33, and 100 ppm ethylene oxide with the control animals exposed to filtered air. Prior to cohabitation, all groups were initially exposed to ethylene oxide for 6 hours/day, 5 days/week for 12 weeks. After 1 week of cohabitation, females with vaginal plugs were removed, and the other females were rotated to a different male to allow for mating for another week. At the end of 2 weeks all male and female animals were separated. The males were then exposed to ethylene oxide for 6 hours/day, 7 days/week for an additional 3 weeks. The females were exposed for 6 hours/day, 7 days/week from day 1 through day 19 of gestation. On day 20 of exposure, females not pregnant were sacrificed. The pregnant females were allowed to deliver and 5 days after parturition were again exposed to ethylene oxide for 6 hours/day, 7 days/week until day 21 postpartum.

The following criteria were used to establish fertility. If a female produced a litter, or if gross examination revealed implantation sites after staining, she was considered fertile. Any female not pregnant after two different matings was considered infertile. If the male impregnated a female after the first mating, then he was considered fertile; any male failing to impregnate a female in two different mating periods was considered infertile. By this criteria, females exposed to 100 ppm had a higher incidence of infertility after mating with a male of proven fertility; however, this incidence did not achieve statistical significance. In the males there was no decrease in fertility. In the 100 ppm group, significantly more females had lengthened gestation periods (time from vaginal plug to birth of litter) than the control, 10, or 33 ppm groups. The control, 10, and 33 ppm groups had gestational periods of 22 days, while the 100 ppm group had gestations ranging from 22-31 days (7/14 rats had 22 day gestation, 4/14 rats had 23 day gestation, 3/14 rats >25 day gestation). Since most of the animals did not have extensively long gestational delays, it is not clear whether this lengthening of gestation represented a true adverse biological effect.

In this study (Snellings et al., 1982a), the number of pups was significantly reduced with a decrease in the number of implantation sites at the 100 ppm level. Of the surviving pups, however, there was no effect on survival after parturition. In the parental generation, there were no adverse effects on body weight or organ histology (testes, epididymides, accessory sex glands, cervix, uterus, ovaries, oviducts, mammary tissues). In the F_1a generation, ≈25% of the animals suffered from sialoacryoadenitis virus infection but this infection appeared to be unrelated to the ethylene oxide exposure.

It was concluded from this study (Snellings et al., 1982a) that ethylene oxide administered to rats by inhalation has the potential to disrupt reproduction by causing an increased incidence of embryolethal effects; however, this embryotoxic effect was only observed when the animals were exposed to the highest dose (100 ppm) and not at the lower doses (10 or 33 ppm) of ethylene oxide.

Snellings et al. (1982b) have also examined teratological aspects by exposing rats by inhalation to 10, 33, or 100 ppm ethylene oxide for 6 hours/day on days 6-15 of gestation. Exposure to 100 ppm caused depression of fetal weight gain, but did not result in fetal death or abnormalities other than variations in ossification of sternebrae and distal thoracic vertebral centra.

9.3.3. Testicular Effects. Hollingsworth et al. (1956) investigated the acute and chronic toxicity of ethylene oxide in a variety of animal species. Positive responses related specifically to the male reproductive system were observed in hamsters and rats. Eight guinea pigs were exposed to 357 ppm ethylene oxide (commercial grade ethylene oxide, 97-98.6% pure by weight) and received 123 seven hour exposures over a 176 day period. There was only moderate growth reduction in the males; however, appreciable degeneration of testicular histology was noted. In another phase of this experiment, both rats and hamsters were exposed to 204 ppm ethylene oxide, 7 hours/day, in 122-157 exposures given over an experimental period of 176-226 days. A slight but not statistically significant decrease in testis weight of rats and guinea pigs was observed. In rats, there was histological evidence of degeneration of the testicular tubules.

A recent study sponsored by NIOSH described the effects of inhaled ethylene oxide on semen production in Cynomologus (Macaca fasicularis) monkeys (Lynch et al., 1983). The monkeys were exposed to 50 and 100 ppm ethylene oxide, (Union Carbide, 99.7% pure) 7 hours/day, 5 days/week for 2 years. In the preliminary range-finding study, only two animals per group was used. Testicular weights were diminished in animals exposed to 100 ppm ethylene oxide but were only marginally decreased in those exposed at the 50 ppm level. Similar decreases in epididymal weights were reported. Sperm motility was significantly reduced at the 50 and 100 ppm level, both in terms of the percentage of motile sperm and the ability of the sperm to travel a given distance in a given time (drive range). In the preliminary study, the sperm concentration was decreased at the 50 and 100 ppm levels. In a subsequent study with larger numbers of monkeys per group (8 or 9), the same types of adverse testicular effects were observed. In this study, there was a 30% decrease in sperm concentration, 30% reduction in motile sperm, and a 3- to 4-fold decrease in distance traveled when the animals were exposed to 50 and 100 ppm ethylene oxide; however, there was no effect on sperm head morphology (Lynch et al., 1983).

In another study relevant to the effects of ethylene oxide on the testis, radiolabeled ethylene oxide was detected in autoradiograms of mouse gonads (epididymis and testis) 20 minutes after intravenous injection (Appelgren et al., 1977). Radioactivity was found in the epididymis up to 24 hours after injection. The results of the dominant lethal mutagenicity test were negative, although inadequacies in this study prevent a firm conclusion from being made (see Section 9.4). This study is relevant to testicular effects because it establishes that ethylene oxide has access to the gonads.

9.3.4. Adverse Reproductive Outcome in Humans. There is little information on the effects of ethylene oxide on the human reproductive system. In one study a comparison was made between the health of 37 male employees involved in ethylene oxide production with 41 men who worked in other production units (Joyner, 1964). This study evaluated many health endpoints including genitourinary problems. The mean exposure period was 10.7 years with a general level of exposure on the order of 5-10 ppm. The range of exposure levels varied from 0-55 ppm. The health survey of the workers considered the following information: 1) the number of sick days taken in a 10 year period with information on the etiology and duration of the illness, 2) any medical diagnosis entered into the medical records and confirmed by an outside physician, 3) any visits to the Medical Division related to respiratory, gastrointestinal, or genitourinary problems. In this study there was a higher incidence of chest abnormalities and a higher incidence of absenteeism attributable to gastrointestinal and genitourinary causes; however, the higher incidence of absenteeism was attributable to a single individual in each category. Therefore, it was concluded that long term exposure to ethylene oxide had no adverse health effect on the men involved in ethylene oxide production. Since this study did not deal specifically with reproductive health problems, it is of limited value in determining the potential of ethylene oxide to cause adverse reproductive effects.

A study by a Russian investigator (Yakubova et al., 1976) reported that female workers involved in ethylene oxide production experienced a number of gynecological and obstetrical problems. These problems included diseases of the cervix, inflammation of the uterus, obstetric anamnesis (this word, as well as others may have been incorrectly translated), hypertonic disease, anemia, toxicosis, and shortened pregnancies. These observations were reported in an anecdotal manner with no presentation of actual data or description of methodologies. This study is therefore of little value in the scientific review of adverse reproductive effects.

Holmberg (1979) and Holmberg and Nurminen (1980) reported case studies of a mother exposed to a variety of organic solvents. These studies describe an adverse reproductive outcome in a woman exposed to alkylphenol and dyes as well as ethylene oxide. It is not clear whether the two articles describe the same woman or two different women. Both reports describe an infant born with hydrocephalus and Holmberg (1979) described a child with additional malformations (cleft palate, double uterus, polydactyly). These reports are not useful in establishing causal relationship between ethylene oxide exposure and congenital malformations because ethylene oxide was not the only chemical involved and because a larger population size would have to be evaluated before such an association can be established.

An epidemiology study has been conducted concerning the effects of ethylene oxide exposure on pregnancy outcomes in nursing personnel. This report is the only one which adequately evaluates the possible causal association between ethylene oxide exposure and adverse human reproductive effects, and has been reviewed in depth by an Environmental Protection Agency epidemiologist (Margosches, 1983). In cooperation with the Finnish investigators, the data have been critically analyzed and reviewed. The following is the text of this evaluation:

> "In November, 1982, K. Hemminki et al. published a study of "Spontaneous abortions in hospital staff engaged in sterilizing instruments with chemical agents" in the British Medical Journal. This study, encompassing all Finnish sterilizing staff at that time,

claimed adjusted spontaneous abortion (s.a.) rates of 16.7% for "exposed" and 5.6% for nonexposed pregnancies among these staff. The report singled out ethylene oxide, glutaraldehyde, and formaldehyde use and suggested concentrations as low as 0.1-0.5 ppm ethylene oxide might have been associated with adverse outcomes. In particular, among hospital-discharge-corroborated pregnancies, the ethylene-oxide-exposed s.a. rate (22.6) significantly exceeded the control s.a. rate (9.2). Also rates among all pregnancies exposed to ethylene oxide or to glutaraldehyde differed significantly from rates among pregnancies not exposed.

This study encompassed staff employed in 1979 at hospitals throughout Finland (including tuberculosis sanitoria and mental hospitals). It was a cohort study looking at past events; determination of exposure status was based on the responses to two questionnaires. The unit for most statistical tabulations and analyses was the pregnancy; while not uncommon in the literature, such a basis cannot take into consideration the relatedness of sibling births or repeated miscarriages of a single woman.

The cohorts, sterilizing staff and controls [hereafter also called group (1) and (2)], were identified by the head nurses at the study hospitals. The former were named in response to a first questionnaire that also queried chemical sterilizing agent use history at each hospital. The latter were obtained as members of cluster samples from auxiliary nurses in departments (not including chemical sterilization, x-ray, or surgery) at the time of distribution of a second questionnaire that focussed on pregnancy and employment history. The investigators obtained a very high return rate (92% among sterilizing staff, 91% among auxiliary nurse controls) and studied the 645 (63%) ever pregnant women among sterilizing staff and the 574 (55%) ever pregnant women among controls. The 17 male sterilizing staff were not studied.

While the study population was selected on the basis of hospital employment as sterilizing staff (1) or non-sterilizing-staff auxiliary nurses (2), Dr. Hemminki classified pregnancies of each group (1) member according to likelihood of exposure and the agent(s) present in order to make finer comparisons. He considered all pregnancies occurring after the first use of ethylene oxide at a hospital to be exposed to ethylene oxide unless an individual did not work at the hospital during a particular pregnancy; similarly for glutaraldehyde and formaldehyde. This was a fairly conservative classification. This study design precluded the examination of the questions whether spontaneous abortions were related to an individual's ever having been exposed to an agent.

Another limiting factor of the study design was the characterization of individual exposures in purely qualitative terms. Dr. Hemminki believes that typical exposures have averaged <1 ppm (measured by gas-tight syringes). He bases this belief on papers published by colleagues at the Institute of Occupational Health covering a 3-year period overlapping the close of the study period, on the unchanged instrumentation of ethylene oxide use over its 20 or so years in Finland, and on the measurement method's 1 ppm detection limit. He did not, however, make any unwarranted inferences regarding possible dose-response relationships. Nevertheless, he did find sizeable differences in adjusted spontaneous abortion rates in both nurses and sterilizing professionals (these are 2 education levels of sterilizing staff) between ethylene-oxide-exposed and non exposed, age-adjusting ($\leq$30) the rates among discharge-registry-identified pregnancies, the ethylene oxide-exposed s.a. rate (16.1) also exceeded the rate (9.4) in control pregnancies but no longer significantly. (Certain of the pregnancies occurring during 1973 to 1979 could be cross-identified through a national hospital discharge register and parallel analyses were carried out on this set and the total questionnaire-obtained set.)

On the whole, this study and its report paid close attention to the possibilities and consequences of such typical epidemiologic afflictions as reporting and recall bias. Additionally, the methodology for statistical analysis, based on rates adjusted for such concomitant variables as age, parity, and decade of pregnancy by logistic regression, is sound (although there may be good reasons to investigate a finer categorization of age). While a "per woman" analysis, the analytic methods for incorporating an individual's pregnancy history have not yet been perfected or standarized.

Unfortunately, the investigators introduced a possible source of bias through telling the supervisory nurses (who identified group (1) and selected group (2)) the purpose of the study, including the names of the agents of interest. Another shortcoming is the impreciseness with which hospital exposure history was determined. Finally, although the authors planned a priori to investigate relationships between ethylene oxide and spontaneous abortions and, possibly, other adverse pregnancy outcomes, the underlying relatedness of multiple pregnancies and of certain of the analyses (e.g., regroupings of the same pregnancies to look at different exposures) dilute the strength of any associations perceived in this study. Notwithstanding these limitations, this work is sufficiently suggestive to support further study of the possible associations between ethylene oxide exposure and adverse pregnancy outcomes or other reproduction effects." (Margosches, 1983).

9.3.5. Summary of Teratogenicity and Reproductive Toxicity. The potential of ethylene oxide to cause teratogenic or adverse reproductive effects has been examined in four animal species (mouse, rat, rabbit, monkey) by two routes of administration (inhalation and intravenous) (Table 9-3).

TABLE 9-3

Summary of Studies on Teratogenicity and Reproductive Toxicity

Type of Study	Route of Administration	Species	Dose Level and Time of Exposure	Findings	References	Comments
Teratology	iv	CD-1 mouse	0, 75, 150 mg/kg day 4-6, 6-8, 8-10 or 10-12 of gestation	1. Developmental toxicity at or near dose level which produced maternal toxicity (150 mg/kg)	LaBorde and Kimmel, 1980	
Teratology	iv	New Zealand White rabbit	0, 18, 36 mg/kg day 6-9 of gestation; 0, 9, 18, 36 mg/kg day 6-14 of gestation	1. Developmental toxicity only at levels which were maternally toxic (36 mg/kg, day 6-14)	Jones-Price et al., 1983	
Teratology/ Reproduction	Inhalation	Sprague-Dawley CD rat	150 ppm, 7 hr/day: day 7-16 gestation, day 1-16 gestation, 3 weeks pregestation plus day 1-16 gestations	Teratology: 1. Retarded fetal development Reproduction: 1. Maternal toxicity 2. Increase intrauterine mortality	Battelle Pacific Northwest Laboratories (NIOSH 210-80-0013) Hackett et al., 1982	Teratology: 1. Inadequacies a) no maternally toxic dose b) dose-response not determined Reproduction: 1. Inadequacies a) dose-response not determined
Teratology/ Reproduction	Inhalation	New Zealand White rabbits	150 ppm, 7 hr/day: day 7-19 gestation, day 1-19 gestation	1. No teratogenic or reproductive effects	Battelle Pacific Northwest Laboratories (NIOSH contract No. 210-80-0013) Hackett et al., 1982	Teratology: 1. Inadequacies a) no maternally toxic doses b) no developmental toxic doses c) dose-response not determined Reproduction 1. Inadequacies a) dose-response not determined

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose Level and Time of Exposure	Findings	References	Comments
*One generation reproduction	Inhalation	Fischer 344 rats	0, 10, 33, 100 ppm 12 wks prior to mating, 6 hr/day, 5 day/wk. During gestation - days 0 through day 19. During lactation - days 5 through 21.	1. No difference in F_0 fertility. No F_0 toxicity. 2. No adverse effects on F_{1a} survival, growth rate, or lactation. 3. Adverse reproductive effects at highest dose, 100 ppm. a) increased gestational length b) decreased litter size c) decreased implantation sites (i.e., decreased fecundity) d) decreased fetuses/ implantation sites (embryo lethal)	Carnegie-Mellon Research Institute, 1979 (Snellings et al., 1982a)*	
Teratology	Inhalation	Fischer 344 rats	0, 10, 33, 100 ppm 6 hr/day, days 6-15 of gestation	1. Depression of fetal body weight (slight)	Snellings et al., 1981b	1. Maternal body weight not monitored during treatment
Chronic toxicity (male reproduction)	Inhalation	Guinea pigs	357 ppm, 123 7-hr exposures in 176 days	1. Tubular degeneration of test with replacement fibrosis	Hollingsworth et al., 1956	
			204 ppm, 122-157 7-hr exposures in 176-226 days	1. Slight decrease in testes weight, not statistically significant.		
		Rats	204 ppm, 122-157 7-hr exposures in 176-226 days	1. Slight decrease in testes weight, not statistically significant. 2. Testes: small, slight degeneration of tubules.		
Testicular toxicity	Inhalation	Cynomologus monkeys	50, 100 ppm, 7 hrs/day for 2 years	1. Decreased testicular weight. 2. Decreased sperm concentration. 3. Decreased sperm motility. 4. No change in sperm morphology.	Lynch et al., 1983	

*A variety of experimental protocols were utilized; only those which provided positive information on reproduction effects are noted here.

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose Level and Time of Exposure	Findings	References	Comments
Medical survey of workers	Occupational exposure	37 male workers	Mean exposure time: 10.7 years. General levels: 5-10 ppm	1. No observed increase in male reproductive disorders.	Joyner, 1964	1. Small sample size 2. Study did not evaluate fertility or testicular function.
Medical survey of workers	Occupational exposure	282 female production workers 259 female management coworkers 100 females controls	<0.2-0.3 mg/m^3	1. Gynecological disorders, spontaneous abortions, toxicosis, decrease birth weights.	Yakubova et al., 1976	1. Difficulties in translated material 2. Little information provided on experimental design. 3. Multiple exposures to noise and high temperatures
Case study	Occupational exposure	Pregnant women		1. Infant with hydrocephalus	Holmberg, 1979; Holmberg and Nurminen, 1980	1. Mother exposed to multiple chemicals 2. Only one infant studied
Epidemiology study	Occupational exposure	Pregnant women	<1 ppm	1. Ethylene oxide exposure associated with an increase in spontaneous abortion	Hemminki et al., 1982	1. Possible bias introduced by supervisors who categorized participants in this study 2. Limited exposure data
Teratology	Intravenous	CD-1 mouse	0, 60, 120 mg/kg on days 4-6, 6-8, 8-10, or 10-12 of gestation	1. Maternal toxicity at 120 mg/kg for all treatment periods 2. Embryotoxicity at 120 mg/kg for all treatment periods and at 60 mg/kg on days 8-10 (fetal weight reduction).	LaBorde and Kimmel, 1980	

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose Level and Time of Exposure	Findings	References	Comments
Teratology	Intravenous	New Zealand white rabbits	0, 9, 18, 36 mg/kg on days 6-14 of gestation	1. No effect on mother or fetus	LaBorde et al., 1982	1. Inadequacies a) no maternally toxic doses b) no developmentally toxic doses
Teratology and Toxicity	Air cell injection	Chick embryo	0, 10, 25, 50, 100 or 200 mg/kg at 0 hour incubation; 0 5, 12.5, 25, 50 or 100 mg/kg at 96 hours incubation	1. Ovo-toxic at levels $\geq$25 mg/kg at 0 hour, and $\geq$12.5 mg/kg at 96 hours. 2. Teratogenic to chick embryo	Verrett, 1974	Uncertainties in extrapolating avian developmental effects to those of mammals

[a] Original study performed by Carnegie-Mellon Research Institute (Bayes, 1979); later published as Snellings et al., 1982a

In a teratology study, Hackett et al. (1982) reported that rats exposed to a single 150 ppm dose of ethylene oxide displayed both maternal toxicity (decreases in food consumption, decreases in body weight, increases in kidney and spleen weights) and toxicity to the developing conceptus (increases in resorptions, decreases in fetal weight, decreases in crown-rump length, and increases in incomplete skeletal ossification). Similar effects were not produced in rabbits exposed to 150 ppm ethylene oxide in this study.

LaBorde and Kimmel (1980) administered 75 and 150 mg/kg ethylene oxide to pregnant CD-1 mice for several gestational intervals. The animals displayed signs of maternal and fetal toxicity at the highest dose level. There were maternal deaths with decreases in the number of implants per litter and an increase in the percentage of malformed fetuses/litter. The malformations appeared to follow a developmental pattern and in at least one gestational interval (days 8-10 of gestation) occurred in the absence of significant observed maternal toxicity.

Similar studies were conducted by Jones-Price et al. (1983) on the effects of 18 and 36 mg/kg ethylene oxide administered intravenously to New Zealand rabbits. Significant maternal toxicity (decreased weight gain) was observed in addition to embryotoxicity observed in the offspring (decreases in the number of live fetus/litter, increases in the number of resorptions/litter). No embryotoxicity was observed in the absence of maternal toxicity.

LaBorde et al. (1982) investigated the teratogenic effect of intravenously administered ethylene chlorohydrin (ECH), a reaction product of ethylene oxide, in CD-1 mice and New Zealand rabbits. No adverse maternal or embryotoxic effects were produced in the rabbits. In the mice at the highest dose (120 mg/kg), however, severe maternal weight loss with increases in resorptions/litter and decreases in fetal weight were observed. At the 60 mg/kg level, with exposure on gestational days 8-10, there was significant fetal weight loss in the absence of maternal toxicity; therefore, the authors concluded that ECH may be a specific hazard to the developing conceptus at this dose level. ECH has also been reported to produce adverse effects in developing chick embryos (Verrett, 1974). Structural abnormalities were produced by 12.5-100 mg/kg of ECH when the egg was incubated with the chemical for up to 96 hours.

In a one-generation study (Snellings et al., 1982a), female rats exposed by inhalation to 100 ppm ethylene oxide had a higher incidence of infertility with indications of a longer gestational period. There was a decrease in the number of pups produced by mothers exposed to 100 ppm ethylene oxide, as well as a decrease in the number of implantation sites. However, there were no significant signs of toxicity in the mothers (no decreases in body weight or changes in organ histology). The same group (Snellings et al., 1982b) observed lowered fetal weights, but not a substantial level of malformations in response to 100 ppm ethylene oxide administered to rats by inhalation on gestation days 6-15.

Adverse effects on the testis resulting from ethylene oxide exposure have been reported for the hamster and rat (Hollingsworth et al., 1956) and Cynomologus monkey (Lynch et al., 1983). Hollingsworth reported testicular degeneration occurring in hamsters and rats exposed to ethylene oxide by inhalation (204-357 ppm). Lynch et al. (1983) reported adverse effects on sperm concentration and motility, but not morphology, in Cynomologus monkeys. The monkeys in this study were exposed over 2 years to 50 and 100 ppm ethylene oxide by inhalation. In mice, radiolabeled ethylene oxide has been found to persist in the epididymis up to 24 hours after a single injection (Appelgren et al., 1977).

Very little information exists on the adverse reproductive effects of ethylene oxide in the human. Medical surveys have described effects ranging from no adverse reproductive outcome (Joyner, 1964) to a variety of adverse outcomes (Yakubova et al., 1976). The study by Joyner (1964) is inadequate because it did not deal specifically with adverse reproductive outcomes. The report by Yakubova et al. (1976) was presented in an anecdotal manner and therefore is of little scientific value. A case report described by Holmberg (1979) and Holmberg and Nurminen (1980) indicated that one women exposed to a variety of substances, including ethylene oxide, produced an infant with multiple defects and hydrocephalus. However, because of the multiple chemical exposures involved, this study is of little value in establishing the potential of ethylene oxide to cause adverse effects.

A recent epidemiological study has been conducted evaluating the pregnancy outcome of nursing personnel exposed to ethylene oxide (Hemminki et al., 1982). Although there were problems in the study design and collection of data, the data are sufficient to suggest an association between ethylene oxide exposure and spontaneous abortion, warranting further examination of adverse pregnancy outcomes. Additional epidemiology studies would be helpful to more firmly establish the potential of ethylene oxide to cause adverse reproductive effects in humans.

In conclusion, ethylene oxide produces adverse reproductive and teratogenic effects in both females (maternal toxicity, depression of fetal weight gain, fetal death, fetal malformation) and males (reduced sperm numbers and sperm motility) if the concentration of the chemical reaching the target organ is sufficiently high or if exposure at lower levels is sufficiently long. Thus, the experiments in which ethylene oxide was injected intravenously have produced more detrimental effects than the short-term inhalation experiments. Even short-term inhalation experiments, however, have resulted in suggestive evidence of detrimental effects. The levels needed to produce the developmental effects approach or equal the levels needed to produce toxicity in the dams. The effects of ethylene oxide on human reproduction have not been studied in depth, although one study indicates that ethylene oxide may be associated with spontaneous abortion (Hemminki et al., 1982). Future studies are needed to establish this effect in humans.

9.4. MUTAGENICITY

Ethylene oxide has been evaluated for mutagenicity in several different systems including tests in bacteria, fungi, higher plants, Drosophila, mammalian cells *in vitro*, and rodents. Effects in humans are also reported. The available data concerning the mutagenicity of ethylene oxide are discussed below and summarized in Tables 9-4 to 9-16. The reader may also wish to refer to other reviews of the mutagenic potential of ethylene oxide (e.g., Fishbein, 1976, Wolman 1979, Ehrenberg and Hussain, 1981, and NIOSH, 1981).

TABLE 9-4

Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutations in Bacteria

Reference	Test System	Strains	Activation System	Chemical Information	Results	Comments
Rannug et al., 1976	*Salmonella*/ microsome assay (suspension assay)	TA1535	None	Concentration tested: 0-95.5 mM Source: Fluka Purity: Not given Solvent: Cold ethanol	Strong positive response	1. Ethylene oxide used as a positive control. 2. Dose-dependent response. 15-fold increase in revertants noted at highest dose compared to negative controls. 3. Five plates used per dose.
Pfeiffer and Dunkelberg, 1980	*Salmonella*/ microsome assay (plate test)	TA98 TA100 TA1535 TA1537	None	Concentration tested: 0-200 µmol/plate (0-8.8 mg/plate) Source: J.T. Baker Chemicals BV Deventer, The Netherlands Purity: 99.7% Solvent: Cold acetone	Positive	1. Dose-dependent response for TA1535 and TA100. 2. Concurrent negative control values not given. 3. Compared to lowest dose (20 µmol/ plate), revertant count at highest dose (200 µmol) was elevated 18-fold for TA1535 and 2.25-fold for TA100. 4. Between 6 and 10 independent runs were done in duplicate for each experiment.

TABLE 9-4 (cont.)

Reference	Test System	Strains	Activation System	Chemical Information	Results	Comments
Tanooka, 1979	*Bacillus subtilis* spores (reversion to his$^+$ prototrophy)	HA 101 (his met leu) TKJ 5211 (his met uvrA10) TKJ 8201 (his met polA151)	None	Concentration tested: 27.3% atmosphere of ethylene oxide gas for times ranging from 5-50 minutes. Source: Daicide LS gas Daido Oxygen Co. Tokyo, Japan Purity: 27.3% ethylene oxide 72.7% Freon	Positive response	1. Tests conducted in a polyethylene bag; 4×10^8 spores placed on sterile filter inside bag. 2. Negative control values not provided. 3. Revertant values expressed as mutation frequency (6×10^{-5} after 5 minutes exposure and 8×10^{-3} after 50 minutes exposure of HA 101 and TKJ 5211). 4. Lethal and mutagenic effects were enhanced in the *polA* strain; TKJ 8201 was 10x more sensitive than HA 101 and TKJ 5211.

9.4.1. Gene Mutation Studies.

9.4.1.1. PROKARYOTIC TEST SYSTEMS (Bacteria) -- Several investigators have shown that ethylene oxide causes point mutations in bacteria (Table 9-4). Ethylene oxide is a very effective sterilant for products that would be damaged by other sterilization methods. Bacillus subtilis var. niger is commonly used to monitor the effectiveness of ethylene oxide sterilization. Jones and Adams (1981) found that treatment of spores of these bacteria with Pennges (12:88 ethylene oxide-Freon mixture by weight) for 5 minutes increased the number of colony variants by five fold over the spontaneous level. Forty aberrant isolates (out of 125 found) were plated five times in succession; of these, 11 reverted to typical appearance, 12 changed to other atypical appearances, and 17 remained stable. Although the changed colonies were not well-defined genotypically these data suggest that ethylene oxide induced mutations in the surviving spores.

In a study by Rannug et al. (1976), ethylene oxide was chosen as a positive control chemical in tests of other chemical substances in the Salmonella/microsome assay. In this study, strain TA1535 was exposed to concentrations of ethylene oxide (purity not reported) ranging from 0-95.5 mM in a suspension test without addition of an exogenous mammalian metabolic activation system (Table 9-4). A statistically significant dose-related response was observed (Figure 9-1) where the maximum killing was ≈20%.

In another Salmonella assay, Pfeiffer and Dunkelberg (1980) exposed strains TA98, TA100, TA1535, and TA1537 to concentrations of ethylene oxide (99.7% pure diluted in cold acetone) ranging from 0-200 µM (0-8.8 mg/plate) (Table 9-4). Between 6 and 10 trials were performed in duplicate. A clear

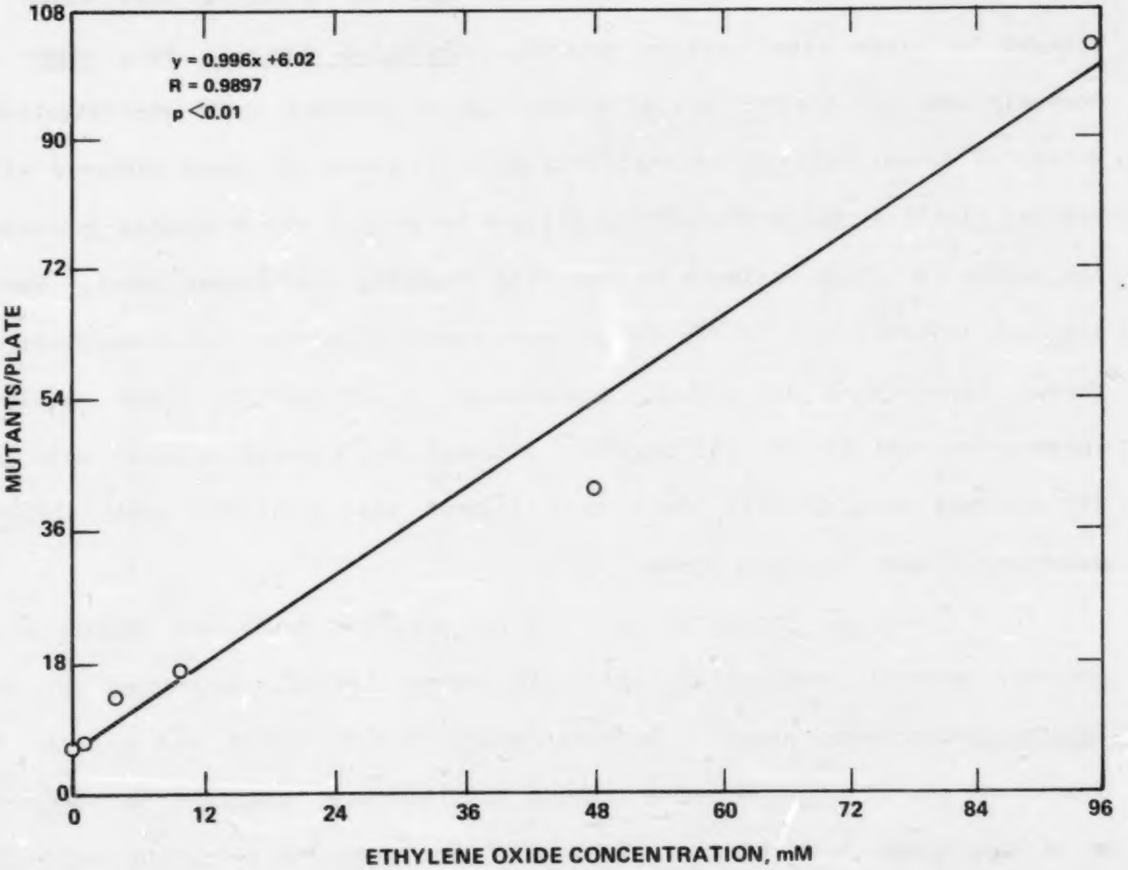


Figure 9-1. Mutagenic response of Salmonella typhimurium strain TA 1535 exposed to ethylene oxide.

Source: Rannug et al. (1976)

dose-dependent response was observed for the base-pair substitution detecting strains TA100 and TA1535 but not for the frameshift detecting strains TA98 and TA1537. This result is consistent with responses observed with other alkylating agents.

Tanooka (1979) exposed spores from three different his⁻ Bacillus subtilis strains to an ethylene oxide gas mixture (Daicide LS, comprised of 27.3% ethylene oxide and 72.7% freon gas) in a plastic bag (Table 9-4). Histidine-independent revertants were selected after treatment; a repair-competent strain and a uvrA repair-deficient strain were treated for times ranging from 5-50 minutes. Exposure-related revertant frequencies were observed for both strains (ranging from 3×10^{-6} after 5 minutes exposure to 2×10^{-4} after 50 minutes exposure). In a similar experiment conducted with a polA strain a significantly higher dose-related revertant rate was reported compared to that obtained with the repair competent and uvrA strains. The revertant frequencies corresponding to 5 and 40 minutes of exposure were about 8×10^{-5} and 3×10^{-3}, respectively. A similarly elevated sensitivity of the polA strain was observed for ethylene oxide-induced toxicity. No data were given for negative controls for any of the strains. The his^+ revertants produced in the repair-competent strain exposed to ethylene oxide gas for 30 minutes were characterized, and 85% of them were found to contain suppressor mutations; 15% were true revertants as measured by cotransformation of $hisB^+$ with the neighboring $trpC^+$ marker using DNA extracted from each his^+ colony. Although this study was not conducted using a "standard" assay system, it does indicate that ethylene oxide is mutagenic in B. subtilis.

The positive responses in these tests show that ethylene oxide causes genetic damage as evidenced by induction of mutations in bacteria. The studies described below show that ethylene oxide causes genetic damage in higher organisms also.

9.4.1.2. EUKARYOTIC TEST SYSTEMS

9.4.1.2.1. Plants -- Kolmark and Kilbey (1968) studied the induction of ad^+ revertants in Neurospora crassa strain K3/17 (macroconidia) after treatment with ethylene oxide (source and purity not given). Five doses ranging from 0.0015-0.15M were employed, but the corresponding mutation frequencies were not reported (Table 9-5). The purpose of the study was to investigate the kinetics of mutation induction. In this study, ethylene oxide was found to be 15-21 times more effective as a mutagen than diepoxybutane.

Migliore et al. (1982) tested a series of aliphatic epoxides for their ability to induce forward mutations in Schizosaccharomyces pombe. Ethylene oxide treatment in liquid suspension at concentrations from 0.5-15 mM resulted in dose-related increases in mutation frequency; survival was reduced ≈60% at the high dose. One hundred-fold increases in mutation frequency were noted at the high dose levels compared to the corresponding negative controls both with and without metabolic activation by phenobarbitone-induced mouse liver S9 mix (50.28 ± 1.76 vs. 0.59 ± 0.22 and 66.21 ± 29.44 vs. 0.66 ± 0.59 mutations/10^4 survivors, respectively). The ranking of the chemical substances tested with respect to their relative specific activity was: epichlorohydrin > ethylene oxide > glycidol > 1,2-epoxybutane > 1,1,1-trichloropropylene oxide > propylene oxide > 2,3-epoxybutane.

Ethylene oxide is known to be a very effective mutagen in higher plants. Many tests have been performed in which ethylene oxide has been shown to be

TABLE 9-5

Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutation Tests in Lower Plants (Yeast)

Reference	Test System	Chemical Information	Results	Comments
Kolmark and Kilbey, 1968	ad-3A revertants in *Neurospora crassa*	Concentration tested: ranged from 0-0.1 M (0-6.2 g/ℓ) ethylene oxide. Source: Imperial Chemical Industries Ltd. Purity: Not given Solvent: Distilled water	Dose-related positive response	1. Objective of work was to study kinetics of mutation. 2. Revertant values given in Figure of paper as mutation frequencies (i.e. $ad^+/10^6$ survivors).
Migliore et al., 1982	Forward mutations at the ade locus in *Schizocaccharomyces pombe*	Source: Montedison (Italy) Purity: 99.70% Solvent: Water and DMSO	Dose-related positive response	

	Without S9		With S9	
Dose (mM)	Survival	Mutation Freq. $.x10^{-4}$	Survival	Mutation Freq. $.x10^{-4}$
0	100	0.66 ± 0.59	100	0.59 ± 0.22
0.5	74.78	1.89 ± 1.00	100	3.32 ± 0.96
1.5	99.19	4.17 ± 0.75	76.64	7.15 ± 0.24
5	80.3	18.77 ± 0.72	100	14.33 ± 7.62
15	35.14	66.21 ± 29.44	42.87	50.28 ± 1.76

mutagenic. The results of these studies will not be analyzed in depth. Most were directed mutagenesis tests conducted to generate desirable traits in food crops. The results of two tests, in which plants were treated with ethylene oxide, will be discussed for illustrative purposes (Ehrenberg et al., 1956; Jana and Roy, 1975). Ehrenberg et al. (1956) administered several chemical substances including ethylene oxide (purity not given) to dry and presoaked barley seeds which were subsequently screened for sterility (dependent on chromosomal aberrations) and chlorophyll mutations (caused by gene mutations, either chromosomal or extrachomrosomal) in the developing plants (Table 9-6). The seeds were exposed to ethylene oxide either as a gas (dry seeds receiving 80% ethylene oxide for 6 days) or in solution [seeds were presoaked in 0.12 and 0.03% (0.27 and 0.07 M) solutions for 2 hours]. Ethylene oxide induced mutations in a dose-dependent manner as can be seen in Table 9-6. A 5-fold increase in lethal mutations and a 33-fold increase in chlorophyll mutations were observed.

Jana and Roy (1975) treated dry seeds of two varieties of rice, IR8 and Dular, with 0.1-0.6% (0.02-0.14 M; pH 7.0)) ethylene oxide (purity not reported) solutions at 10°C for 8 hours. The seeds were sown and the plants grown and harvested. Seeds from single plants were collected and thoroughly mixed to obtain a random sample of seeds. These were then grown to get at least 100 plants from treated original seed for the next generation. These plants were scored for gene mutations affecting chlorophyll expression, and a dose-related mutation frequency was observed (Table 9-6). Although negative controls were not reported, and the spontaneous mutation frequency was not provided, about three times as many mutants were reported in offspring from plants receiving the highest dose compared to those receiving the lowest dose.

TABLE 9-6

Summary of Mutagenicity Testing of Ethylene Oxide: Mutation Tests in Higher Plants

Reference	Test System	Chemical Information	Results	Comments
Ehrenberg et al. 1956	Lethal (chromosomal) and chlorophyll (gene) mutations in barley.	When tested as a gas, resting seeds exposed to 80% ethylene oxide for 6 days. When tested in solution, partly presoaked seeds exposed to 0.03% and 0.12% (0.27 and 0.07 M) ethylene oxide for 2 h at 20°C.	Postive response	1. Third generation progeny not available for analysis when report written; positive response may be due to extra chromosomal mutations. 2. Mutagenic response observed after both types of treatment. 3. Half-life of ethylene oxide in water solution is around 100h at 20°C.

% EtO	% Sterility	% 2nd generation chlorophyll gene mutations	No. spikes analyzed	treatment condition
0	4	0.054	15,861	None
0.03	5.7	0.20	2,510	Solution
0.12	9.5	0.75	1,872	Solution
80	22.1	1.8	989	Gas

TABLE 9-6 (cont.)

Reference	Test System	Chemical Information	Results	Comments
Jana and Roy, 1975	Chlorophyll gene mutations in rice (IR8 and Dular)	Concentration tested: ranged from 0 to 0.6% ethylene oxide. Seeds treated for 8 hours at 10°C and pH 7.0 Source: Eastman Organic Chemicals Purity: Not given Solvent: Not given	Dose-related positive response	1. Objective of study was to study kinetics of mutation. 2. Revertant values given in Figure in text as mutation frequencies.

	% 2nd Generation Chlorophyll Gene Mutations	
%EtO	Dular	IR8
0	---	---
0.1	5.0 ± 0.36	5.9 ± 0.43
0.3	7.0 ± 0.37	7.0 ± 0.30
0.5	12.3 ± 0.32	12.0 ± 0.19
0.6	14.6 ± 0.13	13.1 ± 0.16

The positive responses observed in plants are consistent with the bacterial results and show that ethylene oxide is mutagenic in plants.

9.4.1.2.2. Animals -- Ethylene oxide has also been shown to cause both gene and chromosomal mutations in animals. Bird (1952) injected adult male Drosophila melanogaster (Oregon K) with 0.5 and 0.8% (0.11 and 0.18 M) ethylene oxide to test its ability to induce sex-linked recessive lethal mutations (Table 9-7). The highest dose level approximated the LD_{50}. The exact amount administered and the purity of the sample were not reported. There were no sex-linked recessive lethals in 494 offspring of untreated flies. Ten lethals out of 713 offspring (1.4%) and 9 lethals out of 198 offspring (4.5%) were detected after treatment with 0.5% and 0.8% ethylene oxide, respectively. The dose-related positive response reported indicates ethylene oxide is mutagenic in Drosophila.

Watson (1966) fed ethylene oxide to male Oregon K Drosophila melanogaster to compare the induction of sex-linked recessive mutations with the induction of heritable translocations. A second objective of this study was to compare the effect on mutation yield of storing sperm in seminal receptacles after treatment with alkylating agents. A positive dose-related increase in both endpoints resulted from ethylene oxide treatment (Table 9-7). For the sex-linked recessive lethal test, ≈3% lethals were detected at the low dose (0.4% ethylene oxide) compared to 7% at the high dose (0.7% ethylene oxide). For translocations these values were ≈0.28 and 0.7%, respectively. Negative control values were not given. Storage of ethylene oxide-treated sperm in the seminal receptacles for 6 days had no effect on the frequencies of the two types of genetic damage.

TABLE 9-7

Summary of Mutagenicity Testing of Ethylene Oxide: Gene Mutation Tests in Insects

Reference	Test System	Strain	Chemical Information	Results	Comments
Bird, 1952	*Drosophila melanogaster* sex-linked recessive lethal test	Orgeon K: adult males	Ethylene oxide administered by feeding, inhalation or injection. (Data not presented for first two routes of of administration.) For injection experiments 0.5-5% solutions administered to 20 males. Dosages >0.8% lethal. 0.8% ethylene oxide killed 50% of treated flies while 0.5% ethylene oxide did not affect viability Source: Not given Purity: Not given Solvent: 0.4% saline	Dose-related positive response	1. Objective of experiment was to find most effective method of administration for routine testing. 2. Cannot determine germ cell stage specificity.

% EtO	No. Chromosomes	No. Lethals	% Lethals
0	494	0	0
0.5	713	10	1.4
0.8	198	9	4.5

Reference	Test System	Strain	Chemical Information	Results	Comments
Watson, 1966	*Drosophila melanogaster* sex-linked recessive lethal test and heritable translocation test	Oregon K: adult males	Concentration tested: 0, 0.04, or 0.7% (0, 0.09, or 0.16 M) ethylene oxide Source: Not given Purity: Not given Solvent: Not given	Positive dose-related response	1. Objective of experiment was to determine effect of sperm storage in female seminal receptacle on mutation frequency after treatment with monofunctional and bifunctional alkylating agents. 2. Did not observe storage effect for ethylene oxide with respect to either endpoint. 3. Cannot determine germ cell stage specificity.

	% EtO	%SLRL	% Trans.	% Trans. / % Lethal
Pre-stored	0.4	3.3	0.29	0.08
		3.6	0.39	0.1
	0.7	7.1	0.69	0.1
Post-stored	0.4	3.3	0.79	0.24
		3.1	0.37	0.12
	0.7	6.8	0.60	0.09

TABLE 9-7 (cont.)

Reference	Test System	Strain	Chemical Information	Results	Comments
Lee, unpublished	Drosophila melanogaster sex-linked recessive lethal test and gonadal		Source: Not given for unlabeled ethylene oxide ^{3}H-ethylene oxide from New England Nuclear sp. act. = 2.8 ci/mmole Purity: Not given		1. Objective of experiment was to determine the relation of exposure to level of alkylation of germ cell DNA to mutational response.

Exposure (μmole/25 ml vial)	(Dose) Alkylation/ Nucleotide x 10^{-3}	% SLRL
0	5.58	0.12
0.086	22.3	0.35 ± 0.07
0.43		0.92 ± 0.2

Lee (unpublished data) conducted parallel experiments with unlabeled and ^3H-labeled ethylene oxide to determine: 1) the relationship between exposure and the level of alkylation of germ cell DNA, and 2) the relationship of germ cell DNA alkylation to mutational responses in *Drosophila melanogaster* males.

For both the dosimetry and genetic test treatments, ethylene oxide was given to the flies by adding 0.7 mℓ of cold water solutions to glass fiber paper in 25 mℓ scintillation vials (0.086 or 0.43 µmole/vial). Immediately afterward 50 males were added to the vials which were then sealed. Treatment was continued for 24 hours at 25°C. ^{14}C-Thymidine was also given to males in the dosimetry experiment. The number of alkylations per nucleotide of DNA was calculated based on the $^3H/^{14}C$ ratios in purified sperm DNA (to determine the number of alkyl groups present) and the ^{14}C/sperm cell ratio (to determine the amount of sperm cell DNA in the extraction product). The genetic data showed ethylene oxide to be an effective mutagen since dose-related increases in sex-linked recessive lethals were observed (see Table 9-7). Using the exposure-dose relationship determined from the dosimetry experiments and the genetic data, a doubling dose of 2.3×10^{-3} alkylations/nucleotide was calculated.

These studies show that ethylene oxide is distributed to the gonads of a higher eukaryote (*Drosophila*) and causes heritable genetic damage.

9.4.1.2.3. Mammalian Cells in Culture -- Three tests have been conducted to ascertain the ability of ethylene oxide to cause gene mutations in mammalian cells in culture. Brown et al. (1979) reported in an abstract that polymethacrylate plastic sheets and polypropylene plastic sheets and mesh sterilized by ethylene oxide gas adsorbed ethylene oxide molecules which could be released later to exert a mutagenic effect. They placed the ethylene oxide treated plastic, of unspecified size, in culture flasks containing L5178Y TK+/- mouse lymphoma cells for 3 days. This was followed by dilution in ethylene oxide-free media for 3 days prior to selection using BUdR. Polymethacrylate sheets treated for 18 hours with pure ethylene oxide were estimated to release 8-40 µg ethylene oxide (as measured by GC into the flasks, while similarly treated polypropylene sheets and meshes released 5-100 µg ethylene oxide. Although the spontaneous negative control mutation frequencies were not given, the released ethylene oxide was reported to result in a 2- to 20-fold increase in induced mutation frequency relative to the controls (Table 9-8). It was not possible to evaluate this report critically because it was presented in abstract form.

Tan et al. (1981) administered ethylene oxide (Matheson Co., 99.7% pure, Dr. R. Cumming, personal communication) to Chinese hamster ovary cells at concentrations ranging upwards to 10 mM in the medium. Mutations at the HGPRT locus were selected after 5 hour ethylene oxide treatments both with and without an exogenous metabolic activation system (S9 mix derived from Aroclor 1254-induced rat livers) followed by a 16-18 hour recovery period and subculturing for 1 week. A dose-dependent positive response was obtained at concentrations causing between 10% and 90% cell killing (Figure 9-2) both with and without metabolic activation. The mutation frequency at the highest dose not resulting in excess toxicity (<80% cell killing) was roughly 10 times greater than the reported spontaneous frequency (see Table 9-8).

Hatch et al. (1982) and Dr. S. Nesnow (personal communication, 1983) exposed Chinese hamster V-79 cells to ethylene oxide gas at concentrations up to 7500 ppm and selected for ouabain- and 6-thioguanine resistant mutants. Significant numbers of mutants were produced for both genetic markers. There

TABLE 9-8

Summary of Mutagenicity Testing of Ethylene Oxide: Mammalian Cells in Culture

Reference	Test System	Activation System	Chemical Information	Results	Comments
Brown et al., 1979	L5178Y TK$^{+/-}$ mouse lymphoma gene mutation assay	None	Polymethacrylate (PMMA) plastic sheets and polypropylene (PP) plastic sheets and meshes sterilized for 18 h in pure gaseous ethylene oxide. PMMA retained ethylene oxide and established concentrations of 8-40 µg/20 mℓ cultured medium (1-5 x 10^{-5}M ethylene oxide). PP retained ethylene oxide and established concentrations of 5-100 µg/ 20 mℓ in cultured medium. Source: Not given Purity: Not given Solvent: None	2 to 20-fold induced mutation frequency observed	Presented in abstract. Chemical concentrations measured by gas chromatography. Two ethylene oxide metabolites also tested. At the low, but unspecified, level tested, ethylene glycol residues did not produce an effect. Chlorohydrin produced residues of 15-30 µg/piece of PP. Direct addition of this compound to the medium resulted in a 2-3 x induced mutation frequency.
Tan et al., 1981	CHO-K$_1$-BH$_4$ HGPRT Chinese Hamster Ovary cell gene mutation assay	Liver S9 mix from Aroclor 1254-induced Sprague-Dawley rats	Concentrations tested 0 to 10 mM	Dose-related positive response with and without activation	Concentrations and induced mutants extrapolated from Figure 9-1 of text. 250-300 mutants/10^6 cells at high dose both with and without activation compared to 0-10 mutants/10^6 cells in negative controls. Direct acting mutagen. Ethylene oxide both cytotoxic and mutagenic

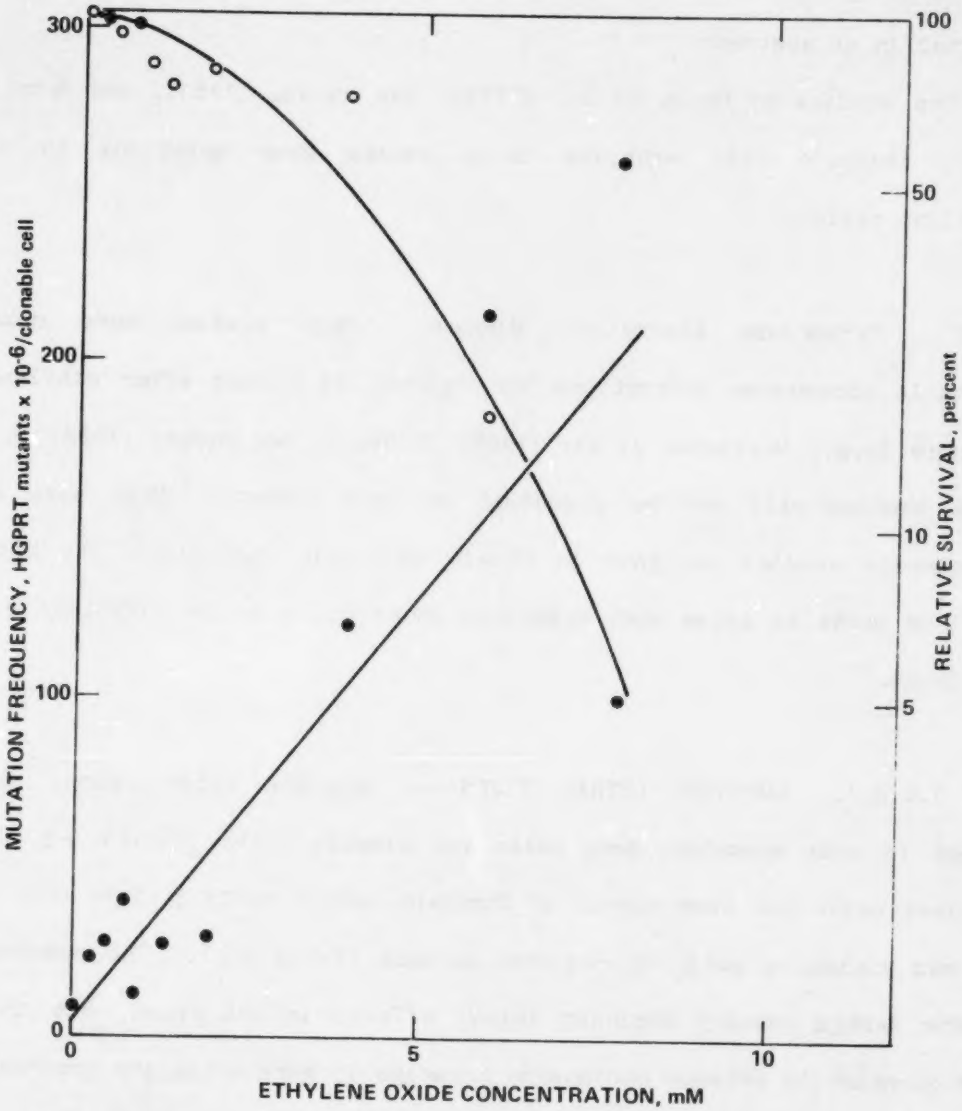


Figure 9-2. Mutagenic response of CHO cells to ethylene oxide.

Source: Adapted from Tan et al. (1981).

was a dose-related increase in mutation frequency. The response at the highest dose was 20 times greater than negative control rates. The work was reported in an abstract.

The studies by Brown et al. (1979), Tan et al. (1981), and Hatch et al. (1982) indicate that ethylene oxide causes gene mutations in cultured mammalian cells.

9.4.2. Chromosome Aberration Studies. Many studies have shown that heritable chromosome aberrations are induced in plants after ethylene oxide exposure [e.g., Moutschen et al. (1968) in barley and Mackey (1968) in wheat]. These studies will not be discussed in this report. Most were directed mutagenesis studies designed to obtain desirable variants. The ability of ethylene oxide to cause such mutations shows it to be an effective clastogen in plants.

9.4.2.1. DOMINANT LETHAL TESTS -- Ethylene oxide causes chromosome damage in both mammalian germ cells and somatic cells (Tables 9-9 to 9-13). Ethylene oxide has been tested in dominant lethal tests in both rats and mice and has yielded a positive response in each (Table 9-9). The precise nature of the damage causing dominant lethal effects is not known, but there is a good correlation between chromosome breakage in germ cells and dominant lethal effects (Matter and Jaeger, 1975). When dominant lethal effects are observed in the offspring of treated males, it can be concluded that the test agent reached the gonads and likely caused genetic damage. Embree et al. (1977) conducted a dominant lethal test with Long Evans rats. Twelve-week-old males inhaled 1000 ppm ethylene oxide for 4 hours (Matheson Gas Products, Newark,

TABLE 9-9

Summary of Mutagenicity Testing of Ethylene Oxide: Dominant Lethal Tests

Reference	Test System	Mating and Sacrifice	Chemical Information	Results	Comments
Embree et al., 1977	Dominant lethal assay in Long Evans rats	Each male placed with 2 virgin females per week for 10 weeks. Females sacrificed on the 17th day after first exposure to male.	12 week old male animals exposed to 1000 ppm ethylene oxide via inhalation for 4 hours Source: Not given Purity: Not given	Positive response. Significant increase in postimplantational fetal deaths during first 5 weeks of the experiment % Dead Implants Week / EtO / Control 1 / 12* / 2 2 / 30* / 10 3 / 30* / 4 4 / 9 / 8 5 / 10* / 4 10 / 9 / 11 *P<0.05	1. Animals exhibited toxicity but no deaths resulted. 2. Pattern of positive response indicates postmeiotic effect.
Generoso et al., 1980	Dominant lethal assay: male mice T stock (Experiment I) and (101 x C3H)F_1 (Experiment II)	Experiment I: Mated to 2 virgin (SEC x C57B1)F_1 females about 12 weeks old. Females replaced when vaginal plug observed. Sacrificed 12-15 days later. Experiment II: Mated to 2 virgins from one of the following stocks T, (SEC x C57BL)F_1, (101 x C3H)F_1, or (C3H x C57BL)F_1. Sacrifice 12-15 days after observation of vaginal plug.	Single i.p. injection of 150 mg/kg. Maximum volume of 1 mℓ Source: Eastman Kodak Co. Purity: Not given Solvent: Double-distilled water	Positive response observed for days 2.5-11.5. Corresponds to treated spermatozoa and late spermatids. During this period 12 to 31% dead implants in treated group compared to 3 to 5% dead implants in negative control group. Little or no difference in the yield of dominant lethal mutations in male postmeiotic germ cells when mated to females from different stocks.	1. i.p. route of administration chosen to mimic implanation of medical device.

TABLE 9-9 (cont.)

Reference	Test System	Mating and Sacrifice	Chemical Information	Results	Comments
Appelgren et al., 1977	Dominant lethal assay: mice	Males mated to 3 virgin females per week. Females sacrificed on 17th day after first exposure to a male.	Single injection of either 0, 0.025, 0.05, or 0.1 g/kg of ethylene oxide given i.v. Source: Not given Purity: Not given Solvent: Saline	Negative response	1. Reported data of dominant lethal test from work by Bateman. 2. Positive controls showed a significant dose-related positive response. 3. Highest dose is 1/3 that used by Generoso et al., 1980; route of administration different from those used by Generoso et al. and Embree et al. (1977). 4. Conducted whole body autoradiography study. Determined ethylene oxide distribution to various tissues in the body, including gonads after either inhalation or injection.

TABLE 9-10

Summary of Mutagenicity Testing of Ethylene Oxide: Heritable Translocation Test

Reference	Test System	Strains	Chemical Information	Results	Comments
Generoso et al., 1980	Heritable translocation	T stock males treated and mated to (SEC x C57BL)F_1 females	Single daily intra-peritoneal injection of 0, 30, or 60 mg/kg of ethylene oxide weekdays for 5 weeks	Dose-related positive response	1. **Shape of response curve consistent with dose-squared kinetics.** 2. **Demonstrates capability of ethylene oxide to cause heritable genetic damage in mice *in vivo*.**

Dose (mg/kg)	Translocation Heterozygotes Frequency	%
0	0/822	0
30	6/456	1.32
60	38/406	9.36
60	6/72	8.33

TABLE 9-11

Summary of Mutagenicity Testing of Ethylene Oxide: Chromosome Aberration Tests

Reference	Test System	Chemical Information	Results	Comments
Fomenko and Strekalova, 1973	Chromosomal aberrations in bone marrow from rats	Concentration tested: 0.001-0.003 and 0.030-0.060 mg/liter for 2, 4, 8, and 30 days by inhalation Source: Not given Purity: Not given Solvent: Not given	Time-dependent positive response at highest dose	1. Method of preparing cells for analysis not given. 2. Criteria for scoring aberrations not given. 3. Definition of terms not given. 4. Insufficient information for adequate evaluation of results.
Strekalova, 1971	Chromosome aberrations in bone marrow from random bred white rats	Concentration tested: 9 mg/kg per os	Positive response reported	1. Animals killed 24 and 48 hours after treatment. 2. Chromosome preparations made from bone marrow squashes. 3. Criteria for classification of aberrations not defined. 4. Insufficient information for adequate evaluation of results.
Poirier and Papadopulo, 1982	Chromosomal aberrations in the human amniotic cell line FL.	Source: Matheson Gas products Purity: Commercial Grade	Dose-related positive response	1. 1 hour vapor exposure. 2. Selected data presented only for cells harvested 72 hours after exposure.

EtO Dose (mM)	% Abnormal Metaphases	Chromatid aberrations/100 cells Breaks	Chromatid aberrations/100 cells Exchanges	% Survival
0	10.8	3.0	5.4	100
5	21.7	15.0	5.0	58
7.5	59.7	37.6	45.5	25
10	77.8	79.2	115.1	9.2

TABLE 9-12

Summary of Mutagenicity Testing of Ethylene Oxide: Micronucleus Tests

Reference	Test System	Chemical Information	Results	Comments
Appelgren et al., 1978	Micronucleus test: NMRI mice and Sprague-Dawley rats	Concentration tested: 0 to 0.3 g/kg (mice) or 0 to 0.2 g/kg (rats) via intravenous injection 30 and 6 hours before the animals are killed. Source: Not given Purity: Not given Solvent: Cold water	Dose-dependent response in mice. Increased incidence in rats, but severe bone marrow depression prevented further characterization.	1. The animals given the highest doses died after the first or second injection. 2. 1000 polychromatic erythrocytes screened for micronuclei per animal.
Conan et al., 1979	Micronucleus test: Swiss mice	Concentration tested: Two injections. Doses ranged from 0-200 mg/kg for i.p. injection, or 0-5 mg adsorbed to implanted plastic devices. Source: Not given Purity: Not given Solvent: Water	Dose-dependent positive response after i.p. injection.	
Jenssen and Ramel, 1980	Micronucleus test: CBA mice (males)	Concentration tested 0-175 mg/kg Source: Fluka AG, Switzerland Purity: Not given Solvent: Not given	Positive response	1. Two-fold increase noted in micronucleus formation (0.33 ± 0.10 in controls compared to 0.93 ± 0.31 at 150 mg/kg.

TABLE 9-13

Summary of Mutagenicity Testing of Ethylene Oxide: Chromosome Mutations in Human Populations

Reference	Test System	Chemical Information	Results	Comments
Thiess et al., 1981	Chromsome aberrations: peripheral blood of occupationally exposed workers		Mutagenic effect indicated	1. Workers were exposed to other alkylene oxides besides ethylene oxide. Cannot assign damage to one agent.
		Exposure:	Aberrations excluding gaps:	
		1. Long-term (>20 years)	1. a. 3.5 b. 2.7	
		2. <20 years	2. 2.3	
		3. Long-term plus accident	3. 2.2	
		4. Accident	4. 1.4	
		5. Control	5. a. 1.4 b. 1	
Pero et al., 1981	Chromosome aberrations: peripheral blood lymphocytes from ethylene oxide-exposed workers	Exposure levels: 0.5-1.0 ppm in air	Suggestive positive response for aberrations excluding gaps. Noted only in comparison	1. Both exposed groups had significantly higher levels of total aberrations (breaks and gaps) compared to the control group

California, purity not given). The LC_{50} is reported to be 1462 ppm for 4 hours. Embree et al. (1977) reported signs of toxicity after treatment but no deaths. Immediately following treatment, each male was mated to two virgin females per week for 10 weeks. The females were sacrificed 17 days after they were caged with a treated male. Statistically significant ($P<0.05$) increases in the number of postimplantation deaths were observed on weeks 1, 2, 3, and 5 after treatment, but not on other weeks, indicating that ethylene oxide exerts its effects on post-meiotic cells. It should be noted that the statistical significance of the increases observed for weeks 1 and 5 may have been due to low negative control values for the corresponding weeks.

Generoso et al. (1980) also observed an increased incidence in postimplantation deaths in mice during the first 2 weeks after administration of 150 mg/kg ethylene oxide (Eastman Kodak, purity not reported) by a single intraperitoneal injection. One dose of 200 mg/kg ethylene oxide was shown to kill 10 of 12 mice. The testing for dominant lethal effects in this study was done in two ways. In the first experiment, T stock males treated with ethylene oxide were mated to two virgin (SEC x C57BL)F_1 females. When a female was impregnated, as evidenced by the observation of a vaginal plug, she was replaced with another female. These females were also replaced after the observation of a vaginal plug and so forth for three weeks post-treatment. The females were sacrificed 12-15 days after the observation of the vaginal plug and were dissected to determine the frequency of dominant lethal effects. A significant increase in postimplantation deaths was observed in females that were bred with treated males between days 2.5 and 11.5 post-treatment (from 12-31% dead implants in treated group compared to 3-5% dead implants in negative control group). This indicates that late spermatids and spermatozoa are sensitive to the test compound. In the second experiment $(101 \times C3H)F_1$ males were injected with ethylene oxide and divided equally into four groups. Four days post-treatment they were mated either to T stock, $(SEC \times C57BL)F_1$, $(101 \times C3H)F_1$, or $(C3H \times C57BL)F_1$ females. The females were checked for vaginal plugs each morning until the 8th day post-treatment and were killed for uterine analysis 12-15 days after the observation of a vaginal plug. The purpose of this experiment was to determine whether the different stocks of mice differed with respect to the ability of oocytes to repair genetic damage induced in the treated male genome. The results of this experiment were consistent with those of the first experiment in showing an increased incidence of postimplantation deaths. However, no significant difference was observed when $(101 \times C3H)F_1$-treated males were mated to females of different stocks.

Appelgren et al. (1977) studied the whole-body distribution of radiolabeled ethylene oxide in mice and reported the results of a dominant lethal test. Male mice were treated with $[^{14}C]$ ethylene oxide (specific activity not given) by inhalation or intravenous injection. The animals were later sacrificed and autoradiograms of midsagittal sections were prepared. The autoradiograms from mice that inhaled ethylene oxide differed qualitatively from those that received the material intravenously in only one respect: the mucosal membranes of the respiratory tract of animals that inhaled the compound accumulated ethylene oxide. In experiments conducted using the intravenous route of administration, ethylene oxide was present in the gonads (epididymis and testicle) 20 minutes after administration. Radioactivity was still present in the epididymis 24 hours after injection.

These observations that ethylene oxide reaches the gonads are consistent with the positive dominant lethal responses reported by Embree et al. (1977) and Generoso et al. (1980). However, the results of the dominant lethal test cited by Appelgren et al. (1977) were negative, in that there was no increase in the incidence of dominant lethal mutations. The highest dose used in this study was 100 mg/kg, as compared to the 150 mg/kg used by Generoso et al. (1980). Since the chemical was administered by intravenous injection in the study by Appelgren et al. (1977) and intraperitoneally by Generoso et al. (1980), it is not clear whether the apparently negative response in the study of Appelgren (1977) is attributable to the difference in the dose or to other factors.

The positive dominant lethal tests reported by Embree et al. (1977) and Generoso et al. (1980) indicate that ethylene oxide reaches the germinal tissue in intact mammals and causes genetic damage. Although these tests do not unambiguously demonstrate heritable effects caused by ethylene oxide, the positive heritable translocation test reported by Generoso et al. (1980) does. Mouse-specific locus tests, which measures heritable gene mutations, are now underway at Oak Ridge National Laboratory and Research Triangle Institute, and the results should provide additional insight into the ability of ethylene oxide to cause heritable mutations in intact mammals.

9.4.2.2. HERITABLE TRANSLOCATION TEST -- In conjunction with their study of dominant lethal effects, Generoso et al. (1980) tested ethylene oxide for its ability to cause heritable translocations in mice (Table 9-10). T stock male mice were given 0, 30, or 60 mg ethylene oxide per kg once daily, weekdays, for 5 weeks. Immediately after the last injection each male was caged with three (SEC x C57BL)F_1 females. After 1 week the treated males were removed, and the females were separated from each other. In the control group, each male was left with one of the three females for ≈5 months after the first litters were born in order to produce additional progeny. The incidence of heritable translocations was as follows: negative control, 0%; 30 mg/kg, 1.32%; and 60 mg/kg, 9.36%. These positive results demonstrate that ethylene oxide causes heritable chromosomal mutations in whole mammals.

9.4.2.3. CHROMOSOME ABERRATION TESTS -- The ability of ethylene oxide to cause well-defined chromosomal aberrations (breaks, rings, inversions, translocations, etc.) has been studied by several investigators. Some of these studies have been discussed above, including the positive heritable translocation tests (Watson, 1966 and Generoso et al., 1980), and work conducted with plants (e.g., Jana and Roy, 1975). Two additional experimental studies were evaluated (Table 9-11). One was by Fomenko and Strekalova (1973), who administered from 0.001-0.003 mg/ℓ or from 0.030-0.060 mg/ℓ ethylene oxide (purity not reported) by inhalation for 2, 4, 8, or 30 days to white rats (strain unspecified). A time-related increase in total aberrations in bone marrow cells was noted in the high dose group (7.1-11.6%) compared to the negative controls (3.0%). The significance of these results cannot be determined, however, because of deficiencies in reporting how the chromosomes were prepared and in defining criteria for scoring aberrations.

Similarly, Strekalova (1971) reported that administration of one 9 mg/kg dose of ethylene oxide per os in aqueous solution resulted in an increased incidence of total aberrations in bone marrow cells scored 21, and in some cases, 48 hours later; the vague manner in which the study is reported, however, precludes an independent evaluation of the results. The most notable problem is that the terms and the criteria for scoring aberrations are not defined. Furthermore, bone marrow squashes were used to prepare metaphase chromosomes for analysis. This technique is not suitable, because it does not yield high quality chromosome spreads compared to chromosome preparations made by the air-drying technique.

Poirier and Papadopoulo (1982) exposed F1 cells (derived from human amnios) to ethylene oxide (commercially available from Matheson Gas Products) at 5, 7.5, and 10 mM for 1 hour. The corresponding number of cells surviving was 58, 25, and 9.2%. Three separate experiments were performed. After harvesting (at 48, 72 and 196 hours) and slide preparation, 150 metaphases were scored for each dose and fixation time (50 from each experiment). Dose-related increases in chromatid aberration were found. For example, at 48 hours after treatment the frequency of exchanges (triradials, 'dicentric' and 'centric' rings) per 100 cells was 5.9, 10.6, 56.7, and 127.3 for the corresponding treatments of 0, 5, 7.5, and 10 mM ethylene oxide/1 hour exposure (Table 9-11).

Ethylene oxide at 50 and 100 ppm 7 hours/day, 5 days/week for 104 weeks also significantly increased the frequency of chromatid/chromosomal aberrations in peripheral lymphocytes of male Cynomologus monkeys (Lynch et al., 1982a; Dr. D. Lynch, personal communication, 1983). The response was dose-related; roughly 4-fold increases in cells with one or more chromatid and/or chromosome aberrations were noted in the high dose animals compared to the negative controls.

9.4.2.4. MICRONUCLEUS FORMATION -- Three studies addressed the ability of ethylene oxide to induce micronuclei (Table 9-12). Appelgren et al. (1978) treated NMRI mice by intravenous injection with two doses of ethylene oxide ranging from 50-300 mg/kg, 30 and 6 hours before sacrifice, and Sprague-Dawley rats according to the same regimen with doses up to 200 mg ethylene oxide/kg. Mice given 300 mg/kg died after the first injection. Rats given 200 mg/kg died after the second injection. In mice, ethylene oxide caused a highly significant dose-related increase in micronuclei. At the highest dose there were 2.48% polychromatic erythrocytes with micronuclei compared to 0.52% polychromatic erythrocytes with micronuclei in the negative control animals ($P<0.001$). Rats also exhibited a statistically significant increase in micronuclei, but it was not shown to be dose-related. Toxicity to the bone marrow confounded the results. The mid-dose level caused 1.08% polychromatic erythrocytes with micronuclei compared to 0.49% polychromatic erythrocytes with micronuclei in the negative controls ($P<0.05$).

Using male Swiss mice, Conan et al. (1979) conducted three different types of experiments to assess the ability of ethylene oxide or its metabolites, ethylene glycol and 2-chloroethanol, to cause micronuclei. Ethylene glycol and 2-chloroethanol were given to the experimental animals via oral administration or intraperitoneal injection. Ethylene oxide was administered by intraperitoneal injection, intravenous injection or intraperitoneal implantation of gas sterilized medical devices. Implantation of the ethylene oxide gas sterilized medical devices did not induce elevated numbers of polychromatic erythrocytes with micronuclei. Similarly, when ethylene oxide was injected intravenously (two injections of 100 mg/kg 24 hours apart) and the animals killed 6 hours after the second injection, no statistically significant increase in micronucleus formation was observed after treatment. However, when ethylene oxide was given intraperitoneally a suggestive positive response was observed. As the dose of ethylene oxide increased from 0-4000 mg/kg (intraperitoneal), the percentage of polychromatic erythrocytes with micronuclei increased from 0.23-0.47.

Jenssen and Ramel (1980) used CBA male mice in their assessment of the ability of ethylene oxide to cause micronuclei. Ethylene oxide was administered intraperitoneally at dosages up to 175 mg/kg, and micronuclei were scored in polychromatic erythrocytes 24 hours later. The response was not clearly dose-related, but a two-fold increase in micronuclei was observed in the animals at the two highest doses (150 and 175 mg/kg) compared to values for the negative control animals (0.93 ± 0.31% and 0.66 ± 0.19% compared to 0.38 ± 0.10%, respectively).

The positive responses obtained in the micronucleus tests of Appelgren et al. (1978) and of Jenssen and Ramel (1980) indicate that ethylene oxide reaches bone marrow and exerts a chromosome damaging (breakage and/or nondisjunction) effect on hematopoietic cells of mammals.

9.4.3. Chromosome Mutations in Human Populations. Three studies have been conducted in which workers exposed to ethylene oxide have been monitored for the presence of chromosome damage in peripheral blood lymphocytes.

Ehrenberg and Hallstrom (1967) monitored eight workers for the presence of chromosome aberrations in peripheral lymphocytes eighteen months after an acute exposure to high, but unspecified, concentrations of ethylene oxide. Ten unexposed persons were selected as controls. The two groups were not characterized in the report and it is not known how well the control group was matched to the exposed group. No analyzable cells were obtained from one person in the exposed group. All samples were coded and an average of 20 metaphase plates was analyzed per remaining person (range = 6-26). Gross chromosome aberrations (i.e., chromosome and chromatid breaks and exchanges, supernumerary chromosomes, and one case of endoreduplication) were elevated in the exposed subjects (17.5%) compared to the unexposed control subjects (4.3%). Chromosomal effects such as this are potentially heritable and represent clear evidence of genetic damage. The addition of chromosome gap data to these values increased the respective incidences to 30.2 and 16.5%. Because of the small size of the study population and the low number of metaphase spreads analyzed, the discriminating power of the study was not great, and, thus, the elevated levels of chromosome damage observed in the exposed population was judged not to be a significant positive effect.

Thiess et al. (1981) monitored 43 humans exposed to ethylene oxide and, to a lesser extent, other alkylene oxides for the presence of chromosomal aberrations (Table 9-13). The workers ranged in age from 27-63 years ($\bar{x}$ = 47.1 years). Individuals were divided into four groups based on the type and extent of ethylene oxide exposure they had received:

1. Long-term exposure (>20 years), 11 men
2. Less than 20 years of exposure, 6 men
3. Long-term exposure plus accident, 21 men
4. Accident (i.e., short-term high exposure to ethylene oxide), 5 men

Subjects in the first three groups worked in plants where ethylene oxide was manufactured or processed. Personnel in the fire department or maintenance workers comprised the fourth group. The negative control group included male office and staff workers, none of whom had been exposed to radiation at the time of testing. The age of individuals in the control group ranged from 24-58 years ($\bar{x}$ = 38.6). The workplace was monitored for ethylene oxide by means of spot samples for up to 2-hour periods and for propylene oxide by personal dosimeters for up to 10 hours over 12-hour shifts. Ethylene oxide exposures were normally <5 ppm but were found to rise to 1900 ppm for several minutes during a plant breakdown. Levels of propylene oxide were usually far below the maximum allowable concentration of 100 ppm, but higher concentrations were measured for brief periods. The percentage of aberrant metaphases, excluding gaps, in cells cultured from 70-72 hours at 37°C in two control groups was 1.4 and 1. Based on Fisher exact test analysis of the data, with Yates correction, significantly increased incidences of chromosomal aberrations were observed in Group I individuals (>20 years exposure) compared to the control group upon examination in October 1978 (3.5%, $P<0.005$). An increased incidence of aberrant metaphases was also noted when these individuals were examined again in August 1979 (2.7%, $P<0.05$). No statistically significant increase was observed for the other groups. The significantly increased rate of chromosome aberrations (excluding gaps) in workers exposed to ethylene oxide for >20 years suggests a mutagenic effect. The results do not conclusively indict ethylene oxide as the causative agent, however, because the workers were exposed to other substances (such as ethylene chlorohydrin, ethyleneimine, propylene oxide, etc.) which may have caused or contributed to the effect. Furthermore, it should be noted that the authors may not have used an appropriate statistical test in their evaluation of the data. In performing the Fisher exact test one must assume that one aberration is independent of another aberration. Within individuals this may not be the case. If a person has one aberration he may be more likely to have a second aberration particularly if the damage was induced in a stem cell. If this were the case in the study by Thiess et al. (1981) one of the basic assumptions of the Fisher-Yates test, that of independence of the observations, would not be met. A more appropriate statistical test, therefore, and one which the authors claimed to have used (but did not report) in their analysis, is the Mann-Whitney test. Use of the Mann-Whitney test to compare Group 1 and the control group shows an increased (and perhaps biologically significant) but not statistical difference between the two groups in regard to aberrations.

Pero et al. (1981) also found increased incidences of chromosome aberrations in factory workers exposed to ethylene oxide (Table 9-13). The workers were divided into three groups. One was an unexposed control group and two were exposure groups (i.e., sterilizers and packers) exposed to 50% ethylene oxide and 50% methyl formate gas (0.5-1.0 ppm ethylene oxide) via inhalation. Chromosome breaks and gaps were scored in the peripheral blood lymphocytes from these individuals. Cells were cultured for 72 hours and 200 metaphases were scored per individual. A statistically significant increase in chromosome gaps plus chromosome breaks was observed in cells from the sterilizer ethylene oxide-exposed group (5 workers) compared to the control group (9 workers), 11-14% in exposed groups compared to 8.5% in controls, ($P<0.05$). With respect to breaks alone, however, a nonsignificant (or at best only a marginally significant) increase was noted in the comparison between sterilizers and control groups (8.2 ± 1.0% compared to 5.8 ± 1.0%, respectively, $P<0.15$). The comparison between the packer (12 individuals), 6.2 ± 0.9%, and control groups was not significant.

The increased incidences of chromosome aberrations in peripheral lympocytes noted in three studies of workers exposed to ethylene oxide are consistent with one another and with the experimental animal data showing ethylene oxide to be clastogenic. They indicate that similar effects are caused in humans as well.

9.4.4. Other Studies Indicative of Genetic Damage. Additional studies have been conducted bearing on the genotoxicity of ethylene oxide (Tables 9-14 to 9-16). These studies do not measure mutagenic events per se in that they do not demonstrate the induction of heritable genetic alterations, but positive results in these test systems do show that DNA has been damaged. Such test systems provide supporting evidence useful for qualitatively assessing genetic risk.

9.4.4.1. SCE FORMATION IN HUMAN POPULATIONS -- Three studies have been reviewed here concerning the induction of SCEs in humans (Table 9-14). Lambert and Lindblad (1980) studied peripheral lymphocytes from five female workers in a German sterilization plant to determine if ethylene oxide exposure causes genotoxic effects in vivo as measured by SCE formation. A description of the exposure these workers received was not reported. The frequency of SCE formation in exposed individuals (19.1%) was increased compared to that of the unexposed control group (14.6%). Although the small sample size and uncharacterized exposure these workers received preclude a definitive assessment of the ability of ethylene oxide to cause SCEs in humans, the results are considered to indicate genetic toxicity in somatic cells of the exposed workers.

TABLE 9-14

Summary of Mutagenicity Testing of Ethylene Oxide: SCE Formation in Human Populations

<table>
<tr><th>Reference</th><th>Test System</th><th>Chemical Information</th><th colspan="4">Results</th><th>Comments</th></tr>
<tr><td>Johnson and Johnson, 1982</td><td>Sister chromatid exchange induction and chromosome aberrations: Industrial workers</td><td>Inhalation exposures estimated to be: Low relative exposure (1 ppm), moderate relative exposure (1-10 ppm), high relative exposure (5-200 ppm).</td><td colspan="4">Dose-response association suggested</td><td>1. Levels of SCE remained elevated after termination of exposure.
2. Environmental exposure to ethylene oxide causes increased SCE formation.
3. Report based on preliminary data from relatively small sample population.</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">SCE
Months after Exposure</td><td colspan="2">Chromosome Aberrations
Months after Exposure</td><td></td></tr>
<tr><td></td><td></td><td></td><td>0</td><td>6</td><td>0</td><td>6</td><td></td></tr>
<tr><td></td><td></td><td>High Potential Exposure</td><td>33</td><td>35</td><td>1.5</td><td>2</td><td></td></tr>
<tr><td></td><td></td><td>Low Potential Exposure</td><td>14</td><td>15</td><td>1.1</td><td>0.9</td><td></td></tr>
<tr><td></td><td></td><td>Inside Controls</td><td>12</td><td>12</td><td>0.6</td><td>0.78</td><td></td></tr>
<tr><td></td><td></td><td>Outside Controls</td><td>--</td><td>8</td><td>--</td><td>0.5</td><td></td></tr>
<tr><td>Garry et al., 1979</td><td>Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers</td><td>Maximum exposures estimated to be 36 ppm (from average measurements over one 8 hour period). Workers divided into groups based on known exposures to ethylene oxide and symptoms indicative of exposure.</td><td colspan="4">Statistically significant increases in level of SCE observed in exposed individuals compared to controls (unexposed laboratory personnel).</td><td>1. Air dried fluorescence plus Giemsa chromosome preps.
2. 20 metphases scored/individual.</td></tr>
</table>

TABLE 9-14 (cont.)

Reference	Test System	Chemical Information	Results	Comments
Yager, 1982 and Yager et al., 1983	Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers	Exposures determined by individually monitoring workers. High exposure group received a cumulative dose >100 mg while cumulative dose for low exposure group was <100 mg.		1. Control group carefully matched to the exposed group for age, sex and personal habits. 2. Exposure estimates based on breathing zone measurements and task frequency estimates.
			Group / Mean Exposure (mg) / SCEs/ cell Control / / 7.56 ± 1.01 Low exposure / 13 / 7.76 ± 1.05 High exposure / 501 / 10.69 ± 1.92	
Laurent et al., 1982	Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers.	No exposure estimates	Exposed group had statistically significant increase in SCEs compared to control group. Range of SCEs for the exposed group was 9.61 - 17.57 compared to a range of 7.04 - 8.52 for the control group.	1. Control group may not have been matched for age, sex, and personal habits to the exposed group.

TABLE 9-15

Summary of Mutagenicity Testing of Ethylene Oxide: SCE Formation in Experimental Studies

Reference	Test System	Chemical Information	Results	Comments
Star, 1980	Sister chromatid exchanges: Cultured human fibroblasts	Concentrations tested: 0 to 3600 ppm and residues from plastic children's endotracheal tubes treated with 1400 mg/cm^3 of pure ethylene oxide for 90 minutes followed by aeration from 24 to 96 hours after sterilization. Source: STERI-Gas cartriges 3M Germany GmbH, Neuss Purity: Not given Solvent: Dulbecco's Modified Eagle's Medium	Toxic as well as mutagenic. Significant increases in SCE induction at 36 ppm. Cytotoxicity at 180 ppm and higher	1. Cultures from skin biopsies used between fifth and tenth subculture. 2. Insufficient data presented to evaluate conclusions.
Yager and Benz, 1982	Sister chromatid exchange induction: New Zealand White rabbits	Concentrations tested: 0, 10, 50, and 250 ppm by inhalation Source: Matheson Dayton, OH	Positive response at 50 and 250 ppm exposures	1. Increased SCE levels decreased after exposure ended but still remained above baseline levels 15 weeks after exposure.
Kligerman et al., 1983	Sister chromatid exchange induction: CDF rats	Concentrations tested: 0, 50, 150 and 450 ppm for 1 or 3 days by inhalation Source: Matheson Gas Product Purity: 99.7%	Dose- and time-dependent positive response Concentration — SCEs/Metaphase 0 — 7.5 ± 0.5 50 ± 7 — 9.1 ± 1.3* 140 ± 17 — 10.3 ± 1.3* 144 ± 33 — 13.6 ± 1.3*	1. Significant increases at 50 ppm show effects induced at levels to which workers have been exposed. Until recently TWA was 50 ppm. 2. Data for 3 days exposure groups shown.

*Significantly different from controls by one-tailed Dunnett's test

TABLE 9-16

Summary of Mutagenicity Testing of Ethylene Oxide: Unscheduled DNA Synthesis

Reference	Test System	Chemical Information	Results	Comments
Cumming et al. (in press)	Unscheduled DNA synthesis: testicular DNA of (101 x C3H)F_1 mice	Concentration tested:		
		a. 600 and 800 ppm for 2, 4, 6, or 8 hours. [^{3}H] dThd administered intratesticularly immediately after ethylene oxide administration	a. Dose-dependent increase in UDS over lower range of doses tested (e.g., 70 dpm/10^6 cells, 48 dpm/10^6 cells, and 8 dpm/10^6 cells for 800 ppm, 600 ppm, and negative controls at 4 hours)	
		b. Same as above except [^{3}H] dThd administered at different times after termination of exposure	b. UDS peaks 2 hours after end of exposure period at day 5 for 300 ppm; at day 1 for 500 ppm	
		c. 300 and 500 ppm 8 hr/day for 5 days. Aliquots of animals sacrificed daily	c. Response peaked at day 5 for 300 ppm; at day 1 for 500 ppm	
		d. 500 ppm for 2, 4, 6, and 8 h. 6 animals given 80 mg/kg 3-methylchloranthrene, 6 animals drank water with 1 mg/ml sodium phenobarbital for 1 week prior to exposure, 6 animals uninduced controls	d. UDS response dramatically reduced in animals receiving mixed-function oxidase inducers	
		Source: Matheson Co., East Rutherford, NJ		
		Purity: 99.7%		
Pero et al., 1981	Unscheduled DNA synthesis: Human lymphocyte cultures	Exposure levels: 0.5 to 1.0 ppm in air	Positive response	1. UDS induced by exposure to N-acetoxy acetyl aminofluorene (NA-AAF). 2. Decreases in NA-AAF-induced UDS measured biochemically and by autoradiography in lymphocytes from ethylene oxide-exposed workers. UDS peaked at 2 mM exposure NAAAF.

In a preliminary, unpublished report Johnson and Johnson (1982) described how they monitored workers at three sterilant facilities for the presence of SCEs and chromosome aberrations in peripheral blood lymphocytes. Based on environmental sampling the workers were assigned to one of the following categories depending upon the plant site at which they worked: high relative exposure (5-200 ppm), moderate relative exposure (1-10 ppm), and low relative exposure (1 ppm). The numerical exposure values represent the estimated range of an 8-hour time-weighted-average inhalation exposure. Employees at each plant were further categorized as to high or low potential for ethylene oxide exposure based on their job description and other factors. During the course of the study it was noted that the SCE levels in the control group of presumably unexposed workers at Plant III were higher than those of other control groups available for comparison at the time (12/metaphase compared to 7/metaphase). The study was therefore expanded to include an additional control group, which was taken from the local community and matched by sex and age to potentially exposed Plant III employees.

Preliminary analysis of the data indicates a consistent dose-response trend at Plant III for SCE induction both at the original monitoring and later after 6 months with no further ethylene oxide exposure (mean values of 12, 14, and 33 SCEs/metaphase for internal controls, low potential exposure and high potential exposure groups, respectively, compared to 8 SCEs/metaphase for the external control groups). A much less pronounced trend was noted at Plant II, and the SCE data for Plant I showed no significant difference between potentially exposed and control groups. Analysis of the chromosome aberration data suggests a dose-related increase in damage, but the magnitude of the differences between groups is not great. Thus, it appears that a dose-response association exists between exposure to ethylene oxide and SCEs in humans and that the increased levels of SCEs appears to be stable, perhaps suggesting long-lived adverse effects caused by human exposure to ethylene oxide. However, it is important to bear in mind that these conclusions are based on preliminary data from a relatively small study population.

In a study of 12 ethylene oxide exposed workers from the instruments- and materials-sterilization areas of a hospital, Garry et al. (1979) reported increased SCE levels in the peripheral blood lymphocytes. The maximum exposure concentration sampled 15 feet from the sterilizer was estimated to be 36 ppm based on an infrared spectroscopy measurement over one 8-hour period during the course of the study. Individuals reporting upper respiratory irritation had statistically significant increases in the incidence of SCEs compared to the control population of 12 unexposed persons working in the adjacent operating room (10.3 ± 1.8 vs. 6.4 ± 0.47, $P<0.01$).

Yager (1982) and Yager et al. (1983) also monitored 14 hospital workers exposed to ethylene oxide. Thirteen persons not exposed to ethylene oxide served as matched controls. Cumulative exposure doses during the 6 months prior to blood sampling were estimated by monitoring air concentrations during defined tasks (using a Wilkes-Miran^{TM} 1A Gas Analyzer) and multiplying this value by the number of sterilizer loads processed. Based on these estimates, the workers were assigned to the low dose group (13 ± 18 mg ethylene oxide) or the high dose group (501 ± 245 mg ethylene oxide). An increased incidence of SCEs/cell was observed in the high dose group (10.7 ± 1.92) compared to the low dose (7.8 ± 1.05) and unexposed control (7.56 ± 1.01) groups.

Laurent et al. (1982) also collected peripheral blood from hospital workers exposed to ethylene oxide. Ten persons in good health and not exposed to any known toxicants were selected as the negative control group. It was not reported whether the controls were matched for sex, smoking habits, etc. They do not appear to have been matched for age because the age of the control group ranged between 20 and 35 years while that of the ethylene oxide exposed workers ranged between 23 and 51 years. No estimate was made of the exposure received by the sterilizer operators, but they had a significantly elevated level of SCE compared to the controls (13.02 ± 2.294 vs. 7.86 ± 0.479).

The increased incidence of SCEs observed in five groups of workers exposed to ethylene oxide does not demonstrate that mutations occurred but does indicate that ethylene oxide can cause genotoxic effects in somatic tissue of humans *in vivo*.

9.4.4.2. SCE FORMATION IN EXPERIMENTAL STUDIES -- Human cells in culture also exhibited increased SCE levels after exposure to ethylene oxide (Table 9-15). Star (1980) exposed skin fibroblast cells from normal healthy human tissue biopsies to 0-3600 ppm ethylene oxide or to plastic children's endotracheal tubes sterilized with 1400 mg/cm^3 ethylene oxide at 55°C for 90 minutes followed by aeration in room air for varying times from 24-96 hours. The cell lines were kept frozen in liquid nitrogen and used between their 5th and 10th subculture. The placement of the plastic tubes in the culture medium resulted in ethylene oxide concentrations ranging from 12-800 ppm as estimated by GC of head space material. Excessive cell killing precluded scoring SCEs above 600 ppm ethylene oxide for the experiment. No statistically significant increase in SCEs was noted in the experiment using the endotracheal tubes, but a consistent, apparently dose-related, rise in SCEs was noted in this part of the study at doses >217 ppm. In the other set of experiments a statistically significant increase in SCE induction was reported at 36 ppm; however, insufficient data were presented to permit an adequate evaluation of the results.

A membrane dosimetry system was developed by Garry et al. (1982) to enable the measurement and determination of dose-response relationships for in vitro exposure to toxic gases. Elevated SCEs were observed in peripheral lymphocytes cultured from healthy humans at exposures as low 10 µg/mℓ (in the media) during a 20-minute exposure period. A dose-related increase was noted up to ethylene oxide concentrations of 35 µg/mℓ (the highest dose tested). At this dose there were about 20 SCEs/cell compared to control levels of ≈5 SCEs/cell.

Yager (1982) and Yager and Benz (1982) administered from 10-250 ppm ethylene oxide gas to 4-month-old male New Zealand White rabbits via inhalation. Eight animals were placed in each exposure chamber for 6 hours/day, 5 days/week, for 12 weeks. Blood samples were taken from the marginal ear vein at 1, 7, and 12 weeks of exposure and 2, 7, and 15 weeks after exposure. Three animals per chamber were used for serial blood sampling for SCE and hematological assays [i.e., red cell count (total and differential), white cell count, hematocrit, and hemoglobin concentration]. One animal was held in reserve and four animals were sacrificed immediately at the end of the 12-week exposure period for analysis of reduced glutathione (GSH) in liver and blood. Positive and negative controls were performed using intraperitioneal injections of mitomycin C and Hanks balanced salt solution, respectively, at each time point. Exposure to 10 ppm did not cause a detectable increase in the incidence of SCEs; however, exposure to 50 and 250 ppm did cause an increase in SCEs (9.47 ± 0.26 and 13.17 ± 0.32, respectively) that decreased after exposure ended, but still remained above baseline levels (7.8 ± 0.23) 15 weeks after exposure (8.45 ± 0.30). Hematological and GSH measurements from the animals did not differ from controls.

After exposures to ethylene oxide of 0, 50, 150, or 450 ppm for 6 hours/day for 1 or 3 days, blood was removed from male CDF rats by cardiac puncture, cultured in the presence of 5-bromodeoxyuridine and scored for SCEs and chromosome breakage (Kligerman et al., 1983). No significant dose-dependent increase in chromosome breakage was observed, but there was a concentration dependent increase in SCEs. Animals in the highest dose group exposed for 3 days had 13.6 ± 1.3 SCEs/cell compared to the control value of 7.8 ± 0.5 SCEs/cell. SCE induction was also significantly elevated after 3 days of exposure to 50 ppm ethylene oxide (9.1 ± 1.3) showing effects in rats at levels to which workers have been exposed. There was no significant reduction in mitotic activity or slowing of cell kinetics.

9.4.4.3. UNSCHEDULED DNA SYNTHESIS -- Cumming et al. (in press) tested ethylene oxide for its ability to cause unscheduled DNA synthesis in germ cells of male mice after inhalation exposures. Four experiments were performed in which hybrid mice (101 x C3H)F_1 were treated with 99.7% pure ethylene oxide (Matheson Co.). In the first experiment, the effect of differential time exposures on unscheduled DNA synthesis induction was assessed. Animals were treated with 600 and 800 ppm ethylene oxide for 2-8 hours, after which exposed animals were anesthetized with metofane and injected intratesticularly with [^{3}H]thymidylic acid (dThd). A dose-dependent increase in unscheduled DNA synthesis was found over the lower end of the dose range for the first 4 hours of exposure in that a higher response was seen at 800 ppm than at 600 ppm (e.g., 70 dpm/10^6 cells for 4-hour exposure at 800 ppm compared to 48 dpm/10^6 for 4-hour exposure at 600 ppm; controls incorporated 8 dpm/10^6 cells). Due to the toxicity of ethylene oxide at 800 ppm it was only possible to measure up to 6 hours exposure for this concentration. In a second experiment, ethylene oxide administration was the same as above, but [^{3}H]dThd was administered to the animals at different times after the end of their ethylene oxide exposure to characterize the unscheduled DNA synthesis response at different times after treatment. Unscheduled DNA synthesis was found to increase with time to a peak 2 hours after the end of the exposure period, and to decrease subsequently. Two additional sets of experiments were performed. The first was a workweek exposure regimen of 300 or 500 ppm for 5 hours/day for 5 days, and the second involved pretreatment of the animals with mixed-function oxidase inducers (either a single intraperitoneal injection of 80 mg/kg 3-methylcholanthrene or administration of drinking water containing 1 mg/mℓ phenobarbital for 1 week prior to ethylene oxide treatment). Concerning the workweek exposures, little effect was noted after the first two exposure periods at 300 ppm. An effect was subsequently noted which rose to a maximum after the 5th exposure period. At 500 ppm the maximum effect was seen after the first exposure period. Apparently, increased levels of DNA damage occurred throughout the week, but after the third exposure period the capacity to respond to this damage appeared to be limited.

Pero et al. (1981, 1982) treated peripheral lymphocytes taken from ethylene oxide exposed workers with 10 mM N-acetoxy-2-acetylaminofluorene (NA-AAF) for 1 hour and subsequently measured the incorporation of [^{3}H] thymidylic acid into DNA to detect unscheduled DNA synthesis (Table 9-16). NA-AAF-induced unscheduled DNA synthesis was found to be inversely related to the duration of worker exposure to ethylene oxide and to the number of chromosome breaks observed. This suggests an inhibition of the cellular DNA-repair capacity by ethylene oxide. Biochemical and autoradiography studies were consistent with this response. When NA-AAF-treated lymphocytes were exposed to ethylene oxide, it was found that concentrations above 2 mM resulted in inhibition of unscheduled DNA synthesis.

As was the case for the studies of SCE induction, these results do not show that ethylene oxide is mutagenic but do indicate it causes damage to DNA and are consistent with the results showing that ethylene oxide causes mutations.

9.4.5. Summary and Conclusions of the Mutagenicity of Ethylene Oxide

Ethylene oxide has been shown to induce gene mutations in bacteria, fungi, higher plants, Drosophila, and cultured mammalian cells in tests conducted without the use of exogenous hepatic metabolic activation systems. It is therefore a direct-acting mutagen. Ethylene oxide has also been shown to induce dominant lethal effects in mice and rats; chromosomal aberrations in higher plants, Drosophila, mice, and rats; and micronuclei in mice and rats. Based on these positive findings in different test systems, ethylene oxide is judged to be capable of causing chromosomal aberrations. It has also been shown to induce SCE in rabbits, rats and humans.

Tissue distribution studies have shown that ethylene oxide reaches the gonads. This result is consistent with evidence that ethylene oxide causes unscheduled DNA synthesis in germ cells of male mice and heritable mutations in insects and rodents (i.e., sex-linked recessive lethals and heritable translocations in Drosophila, dominant lethals in rats and mice and heritable translocations in mice). Ethylene oxide can therefore be regarded as mutagenic both in somatic cells and in germ cells.

Based on the available data, there is overwhelming evidence that ethylene oxide is a direct-acting mutagen that has the potential to cause mutations in the cells of exposed human tissue. The observations that ethylene oxide reaches and reacts with mammalian gonadal DNA, and causes heritable mutations in intact mammals, indicate that it may be capable of causing heritable mutations in man provided that the pharmacokinetics of ethylene oxide in humans also results in its distribution to the DNA of germ cells. Thus, ethylene oxide should be considered a potential human mutagen.

9.5. CARCINOGENICITY

The purpose of this section is to evaluate the likelihood that ethylene oxide is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact and evaluating its potency in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior, have an important bearing on both the qualitative and the quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. The carcinogenicity of ethylene oxide has also been evaluated by the International Agency for Research on Cancer (1976). This section presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and, finally, a summary and conclusions dealing with all of the relevant aspects of the carcinogenicity of ethylene oxide.

9.5.1. Animal Studies. Only a few studies have been conducted to assess the carcinogenicity of ethylene oxide. Most of the reported studies have dealt with subcutaneous administration and skin painting of the compound in mice and intragastric administration in rats. These studies are discussed briefly herein. Two lifetime inhalation studies in rats have been performed (Snellings et al., 1981 and Lynch et al., 1982), and they will be described in detail.

9.5.1.1. MICE -- Reyniers et al. (1964) conducted a study of female germ-free mice that developed tumors (63/83) after being accidentally exposed to ethylene oxide-treated ground-corncob bedding for 150 days, and were moved to untreated bedding for the rest of their lifespans. These animals developed ovarian, lymphoid, and pulmonary tumors. Colony mates maintained on untreated bedding did not develop tumors. All males exposed to ethylene oxide-treated bedding died, with necropsy showing massive hemorrhage. The causative agent was not identified, since chemical analysis of the bedding was not done. The high number of tumors could have been due to other chemicals (such as ethylene glycol or 2-chloroethanol, both derived from ethylene oxide) or to a viral agent, although the authors believed that a viral agent was unlikely. High toxicity is indicated by these findings in male mice. Because germ-free mice are T-lymphocyte deficient, they may be more susceptible than normal animals to tumor development, or the tumor development may be due to immune suppression. At present, however, there is no evidence to support these hypotheses.

Dunkelberg (1979) studied the oncogenic activity of ethylene oxide dissolved in tricaprylin and administered subcutaneously to the interscapular area of groups of 100 female NMRI mice in weekly dosages of 0.1, 0.3, and 1.0 mg. The incidence of spontaneous subcutaneous tumors in these mice was between 0 and 2%. Preliminary results up to the 91st week of treatment showed that 6, 8, and 12 local tumors (sarcomas) occurred in mice receiving total ethylene oxide doses of 9.1, 27.3, and 91.0 mg, respectively. No local tumors occurred in mice receiving no treatment or tricaprylin alone. The number of tumors at sites distant from the injection area was not significantly greater in the group treated with ethylene oxide than in the two control groups. The final report of this study (Dunkelberg, 1981) covers the period from the start of the study to 106 weeks, at which time all of the animals were sacrificed. No increase in tumors at remote sites was observed.

Lifetime skin painting studies with 10% ethylene oxide in acetone (three times weekly) were performed on 30 female mice by Van Duuren et al. (1965). Application of 0.1 mL of ethylene oxide solution to the clipped dorsal skin produced no tumors. Median survival time for the mice was 493 days. The investigators indicated that rapid evaporation of the compound from the skin was responsible for the negative results observed.

9.5.1.2. RATS -- Walpole (1958) injected 12 rats subcutaneously with a maximum total ethylene oxide dose of 1 g/kg (dissolved in arachis oil) over 94 days (dosing schedule not specified). Rats were observed for their lifetimes following treatment, and no tumors were observed. Since the total amount of ethylene oxide administered and the frequency of injection were not specified, it is difficult to evaluate this negative result.

Dunkelberg (1982) administered ethylene oxide intragastrically by gavage at two dosages, 30 and 7.5 mg/kg body weight, to two groups of 50 female Sprague-Dawley rats with empty stomachs twice weekly for a period of nearly 3 years, using salad oil as the solvent. One group was treated with the solvent alone, and the other group was left untreated. A positive control group was treated with β-propiolactone. The test substances were dissolved in 1 mL of oil immediately before treatment. The design of the experiment is summarized in Table 9-17 and the results are summarized in Table 9-18. Ethylene oxide induced local tumors, mainly squamous cell carcinomas of the forestomach. The first tumor occurred in the 79th week. The tumor rates were 62% in the 30 mg/kg group and 16% in the 7.5 mg/kg group. In addition, carcinomas in situ, papillomas, and reactive changes of the squamous epithelium of the forestomach were observed in other animals. An unspecified number of tumors occurred in the glandular stomach. Ethylene oxide did not induce tumors at sites away from the point of administration. Survival decreased in the positive control group.

TABLE 9-17. DESIGN SUMMARY FOR CARCINOGENICITY TESTING OF ETHYLENE OXIDE BY INTRAGASTRIC ADMINISTRATION TO SPRAGUE-DAWLEY RATS

Group	Single dose (mg/kg body wt) (2x weekly)	Average total dose (mg/kg body wt)	Number of animals
Ethylene oxide I	30.0	5112	50
Ethylene oxide II	7.5	1186	50
Oil (vehicle)	1.0 mL	-	50
Untreated	-	-	50
β -Propiolactone	30.0	2868	50

SOURCE: Adapted from Dunkelberg, 1982.

TABLE 9-18. TUMOR INDUCTION BY INTRAGASTRIC ADMINISTRATION OF ETHYLENE OXIDE IN FEMALE SPRAGUE-DAWLEY RATS

Dose	Number of rats with stomach lesions			
	Reactive changes[a]	Carcinoma in situ	Fibrosarcoma	Squamous cell carcinoma
7.5	9	4	0	8
30.0[b]	11	4	2	29

No stomach tumors were seen in either vehicle-controls or untreated controls.

[a] Reactive changes of the squamous epithelium of the stomach comprised hyperkeratosis, hyperplasia, and papillomas.

[b] Fifteen animals from the ethylene oxide I group developed stomach tumors, of which 10 exhibited metastasis and invasive growth into neighboring organs.

SOURCE: Adapted from Dunkelberg, 1982.

Two other studies designed to test for chronic toxicity of ethylene oxide reported no tumors; however, the exposure and observation periods were too short to adequately test the carcinogenicity of ethylene oxide in rats, mice, monkeys, guinea pigs, and rabbits (Hollingsworth et al., 1956; Jacobson et al., 1956).

9.5.1.2.1. Snellings et al. (1981) Inhalation Study -- A 2-year inhalation study (unpublished) was performed by Bushy Run Research Center, Pittsburgh, Pennsylvania (Snellings et al., 1981). Fischer 344 rats were exposed to 100, 33, and 10 ppm of ethylene oxide vapor by the inhalation route, 6 hours/day, 5 days/ week, for approximately 2 years. Two groups were exposed to untreated air under similar conditions. Whole-body exposures were conducted in a dynamic exposure system in which the vapor concentration levels were determined by gas chromatography. Initially, 120 rats per sex per group were exposed, with interim sacrifices of 10 animals each at 6 and 12 months and 20 animals at 18 months to determine possible treatment-related effects. Interim and terminal evaluation included hematology, serum clinical chemistry, urinalysis, body weight, organ weight, bone marrow cytogenetic studies, and gross and histologic examinations.

In the cytogenetic studies, no statistically significant differences were noted for the "percentage of abnormal cells," the "average number of chromosomal aberrations per cell," or the "total number of chromosomal aberrations (per rat)" for either males or females exposed to ethylene oxide at 100 ppm when compared with values obtained for the air-control groups. However, statistically significant chromosomal aberrations have been found in other ethylene oxide studies (see section on mutagenicity).

Histopathologic examination was performed on all tissues of each air-control group and the 100 ppm group at 6 months and at 12- and 18-month necropsy intervals. At 6, 12, and 18 months, for the two lower groups (10 and 33 ppm), this histopathologic examination was performed only when the tissue had gross lesions. At the 24-month necropsy interval, the histopathologic examination was performed on all tissues of rats in the 100 ppm group and both control groups, and on potential target tissues, selected tissues, and tissues with gross lesions in the two lower-dose groups (10 and 33 ppm).

During the 15th exposure month, all rats became infected with sialodacryoadenitis (SDA) virus infection. Clinical signs of infection were noted during the 62nd and 63rd exposure weeks. After the 64th exposure week, the exposures were temporarily terminated to permit recovery from the viral infection. Very low mortality had been observed prior to the infection of the initial 120 rats per sex per exposure group; no more than five in any group of one sex had died or were sacrificed because of a moribund condition. During the 64th and 65th exposure weeks, a total of 24 rats died. There was a higher rate of mortality among female rats in the 100 ppm exposure group than in any other group. Gross and microscopic examination of tissues of the animals that died during this infection period revealed no pathologic findings sufficient to explain the cause of death. Most of the clinical signs associated with the infection subsided after 2 weeks of no exposure, as the mortality rate and body weights returned to preinfection values. As a result, the exposure was restarted. No increase in mortality in association with this disease had been reported in the literature.

According to Snellings et al. (1981), the total numbers of rats that died or were sacrificed in a moribund condition were 49, 39, 28, 31, and 29 for the males and 53, 31, 25, 19, and 20 for the females in the 100 ppm, 33 ppm, 10 ppm, Air Control I, and Air Control II groups, respectively. One additional male in the 33 ppm group and one female in Air Control Group I were accidentally killed.

The cumulative mortality data and statistical significances for male and female rats are shown in Tables 9-19 and 9-20, respectively. The cumulative percentage dying in the 100 ppm group for both sexes was significantly higher than that of controls for at least the last four exposure months of the study. Very few significant differences were observed in males of the 33 ppm group.

During the 15th exposure month, the mortality rate of females in the 100 ppm group increased significantly. This increase was also noted for males in the 100 ppm group and females in the 33 ppm group, but to a lesser degree. Since the SDA virus may have contributed significantly to this mortality, the data were re-evaluated by Snellings et al. (1981), using the number of rats alive at the beginning of month 17 as the starting point. This re-evaluation eliminated the immediate effects of the SDA virus infection. The results of these calculations, presented in Tables 9-21 and 9-22, indicate a significant increase in mortality in the 100 ppm group versus the controls for both males and females, but the increased mortality was not significant until month 23 for the males and month 22 for the females. In no time interval was the cumulative percentage mortality value for either sex in the 33 ppm group significantly different from that of combined controls. However, from the 21st month on, the values for both sexes in the 33 ppm group were higher than those for both control groups. At no time were significant increases in mortality observed in the 10 ppm exposure group of either sex.

Of the many tumor types occurring in the Snellings et al. (1981) study, six types, which may be treatment related, are reviewed here: subcutaneous fibroma, peritoneal mesothelioma, pancreatic adenoma, pituitary adenoma, brain neoplasm, and mononuclear cell leukemia. The authors presented no evidence that the SDA viral infection increased the tumor incidence in the experimental

TABLE 9-19. CUMULATIVE PERCENTAGES OF MALE FISCHER 344 RATS THAT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER EXPOSURE TO ETHYLENE OXIDE VAPOR[a]

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm[b]	10 ppm	Air Control I	Air Control II	Combined controls
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.8	0.0	0.4
6	0.0	0.0	0.0	0.8	0.0	0.4
7	0.0	0.0	0.0	0.8	0.0	0.4
8	0.0	0.0	0.0	0.8	0.0	0.4
9	0.0	0.9	0.9	0.8	0.0	0.4
10	0.0	1.8	0.9	0.8	0.0	0.4
11	0.0	2.8	0.9	0.8	0.0	0.4
12	1.0	2.8	0.9	0.8	0.0	0.4
13	1.0	4.8	0.9	0.8	0.0	0.4
14	3.0	4.8	0.9	1.8	0.0	0.9
15	7.0	6.8	0.9	1.8	2.0	1.9
16	7.0	8.8	1.9	1.8	1.0	1.9
17	7.0	9.8	2.9	2.9	4.1	3.5
18	10.4	9.8	2.9	5.1	5.2	5.2
19	11.7	12.5	2.9	5.1	5.2	5.2
20	18.2	15.1	8.0	9.0	6.5	7.8
21	$24.7^{(-,-,a)}$	20.3	10.6	11.5	10.4	11.0
22	$27.3^{(-,-,-)}$	$29.4^{(-,a,a)}$	14.4	17.9	11.7	14.8
23	$44.2^{(a,c,c)}$	$36.0^{(-,b,b)}$	18.3	21.8	13.0	17.4
24	$50.7^{(a,c,c)}$	$39.9^{(-,a,-)}$	25.9	12.9	20.8	25.2
24.5	$55.9^{(a,b,c)}$	42.5	31.0	34.6	28.6	31.6
25.0	$65.2^{(a,-,b)}$	54.2	38.3	41.9	42.6	42.3

---

[a] Life table analysis, adjusted for scheduled interim sacrifices.

[b] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

$a = 0.05 > p > 0.01$ $b = 0.01 > p > 0.001$ $c = p < 0.001$ - = not significant

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-20. CUMULATIVE PERCENTAGES OF FEMALE FISCHER 344 RATS THAT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER EXPOSURE TO ETHYLENE OXIDE VAPOR[a]

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm	10 ppm	Air Control I	Air Control II	Combined controls
1	0.0	0.0	0.0	0.8	0.0	0.4
2	0.0	0.0	0.0	0.8	0.0	0.0
3	0.0	0.0	0.0	0.8	0.0	0.0
4	0.0	0.8	0.0	0.8	0.0	0.0
5	0.0	0.8	0.0	0.8	0.0	0.4
6	0.0	0.8	0.0	0.8	0.0	0.4
7	0.0	0.8	0.0	0.8	0.0	0.4
8	0.0	0.8	0.0	0.8	0.0	0.4
9	0.0	0.8	0.0	0.8	0.0	0.4
10	1.8	0.8	0.0	0.8	0.0	0.4
11	1.8	0.8	0.0	0.8	0.0	0.4
12	1.8	0.8	0.0	0.8	0.0	0.4
13	2.8	1.8	0.0	0.8	0.0	0.4
14	3.9	1.8	0.0	0.8	0.0	3.0
15	$16.0^{(b,b,b)}$	5.9	2.0	2.8	3.1	3.5
16	$18.0^{(b,b,b)}$	5.9	3.0	3.8	3.1	3.5
17	$21.1^{(c,c,c)}$	6.9	5.0	3.8	3.1	3.5
18	$22.2^{(b,c,c)}$	10.3	6.2	6.1	4.3	5.2
19	$25.0^{(a,c,c)}$	15.5	11.3	8.6	5.6	7.1
20	$30.4^{(b,a,b)}$	16.8	11.3	9.9	12.3	11.0
21	$34.4^{(b,a,b)}$	22.0	12.6	9.9	16.3	13.0
22	$41.3^{(c,b,c)}$	24.6	13.9	9.9	18.9	14.3
23	$49.5^{(c,b,c)}$	32.4	24.2	18.8	22.9	20.8
24	$63.3^{(c,c,c)}$	35.2	28.5	22.9	25.8	24.3
24.5	$70.0^{(c,c,c)}$	41.1	34.7	25.9	25.8	25.9

[a] Life table analysis, adjusted for scheduled interim sacrifices.

[b] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

$a = 0.05 > p > 0.01$ $b = 0.01 > p > 0.001$ $c = p < 0.001$ - = not significant

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-21. CUMULATIVE PERCENTAGES OF MALE FISCHER 344 RATS THAT WERE ALIVE AT THE BEGINNING OF MONTH 17, BUT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER SUBSEQUENT EXPOSURE TO ETHYLENE OXIDE VAPOR[a]

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm[b]	10 ppm	Air Control I	Air Control II	Combined controls
17	0.0	1.1	1.0	1.0	2.1	1.6
18	3.7	1.1	1.0	3.3	3.2	3.3
19	5.0	4.0	1.0	3.3	3.2	3.3
20	12.0	6.8	6.2	7.2	4.6	5.9
21	19.0	12.6	8.8	9.9	8.6	9.2
22	21.8	22.6	12.8	16.4	9.9	13.2
23	40.0(a,c,c)	29.8(-,a,-)	16.7	20.3	11.2	15.8
24	46.9(-,c,b)	34.1	24.5	28.2	19.2	23.7
24.5	52.5(-,b,b)	36.9	29.7	33.4	27.1	30.3
25.0	62.5(-,-,a)	49.8	37.1	40.8	41.4	41.2

---

[a] Life table analysis, adjusted for scheduled interim sacrifices.

[b] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

$a = 0.05 > p > 0.01$ $b = 0.01 > p > 0.001$ $c = p < 0.001$ - = not significant

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-22. CUMULATIVE PERCENTAGES OF FEMALE FISCHER 344 RATS THAT WERE ALIVE AT THE BEGINNING OF MONTH 17, BUT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER SUBSEQUENT EXPOSURE TO ETHYLENE OXIDE VAPOR[a]

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm[b]	10 ppm	Air Control I	Air Control II	Combined controls
17	3.7	1.1	2.1	0.0	0.0	0.0
18	5.1	4.7	3.2	2.3	1.2	1.8
19	8.4	10.2	8.6	5.0	2.6	3.8
20	15.1	11.6	8.6	6.3	9.4	7.8
21	20.1	17.1	9.9	6.3	13.6	9.8
22	28.4(b,-,a)	19.9	11.2	6.3[c]	16.3[c]	11.2
23	38.4(a,-,b)	28.2	21.8	15.5	20.4	17.9
24	55.2(c,c,c)	31.2	26.2	19.8	23.4	21.6
24.5	63.4(c,c,c)	37.4	32.6	22.9	23.4	23.2

---

[a] Life table analysis, adjusted for scheduled interim sacrifices.

[b] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > p > 0.01$ b = $0.01 > p > 0.001$ c = $p < 0.001$ - = not significant

[c] Control I group differed significantly from Control II group at the $p < 0.05$ level only for the 22-month mortality count.

SOURCE: Adapted from Snellings et al., 1981.

groups. The time to first tumor for some neoplasms (but not for mononuclear cell leukemias) was decreased in the high-dose group as compared to controls, as shown in Table 9-23. Median time-to-tumor was not reduced.

Histopathologic examinations were performed on tissues of all the rats in the 100 ppm group and both control groups. In the 33 and 10 ppm groups, only those tissues that had gross lesions were examined. Therefore, some small tumors in these two groups may have been missed, yielding an erroneously low estimate of tumors.

In male rats sacrificed at 24 months, a statistically significant increase in subcutaneous fibromas (10/28, 35.7%) was observed in the group exposed to 100 ppm ethylene oxide as compared with combined controls (3/91, 3.3%) (Table 9-24). An increased prevalence of these tumors was also observed in the 10 ppm group (8/48, 17%); however, this increase was not significant. No increase in subcutaneous fibromas was observed in the 33 ppm group. The authors concluded that the increased prevalence of subcutaneous fibromas in the 100 ppm group represented an effect of treatment. It should be noted, however, that histologic examinations were performed only on skin sections that showed gross lesions; therefore, many tumors too small for gross detection were probably missed. When the incidences of this tumor type were added to those for animals that died spontaneously or were euthanized when moribund, the totals were even higher in both the 100 and 10 ppm groups than in the controls (Table 9-25).

An increase in the frequency of peritoneal mesothelioma was observed in all of the male treatment groups sacrificed at 24 months (4/30 at 100 ppm, 4/39 at 33 ppm, 2/51 at 10 ppm vs. 1/48 for the Control I group and 2/84 for the Control II group) (Table 9-24). Although the increase was not significant at any dose level, this enhanced prevalence in the 100 and 33 ppm groups is considered a treatment-related effect. This tumor was also found in a large

TABLE 9-23. SUMMARY OF SELECTED TUMOR INCIDENCE COMPARISONS FOR MALE AND FEMALE FISCHER 344 RATS EXPOSED TO ETHYLENE OXIDE FOR 2 YEARS

Ethylene oxide concentration ppm	Total number of rats		Time in months to:	
	With tissues examined	With tumor[a]	First tumor	Median tumor[b]
Mononuclear cell leukemia - Males				
100	119	26	19	24
33[c]	81	25	13	25
10[c]	79	21	20	25
0-I	116	20	18	23
0-II	118	18	21	25
Mononuclear cell leukemia - Females				
100	113	28(c,b,c)	18	24
33[c]	79	24(c,c,c)	18	24
10[c]	77	14	19	25
0-I	118	9	19	24
0-II	117	13	18	23
Peritoneal mesothelioma - Males				
100	119	22(c,c,c)	15	23
33[c]	91	7(a,a,a)	18	25
10[c]	89	3	20	--
0-I	114	2	18	--
0-II	116	2	20	--
Pituitary adenoma - Males				
100	117	27	15	25
33[c]	79	16	15	25
10[c]	80	27	18	25
0-I	117	28	17	25
0-II	117	22	18	25
Pituitary adenoma - Females				
100	117	32	10	24
33[c]	90	38	17	25
10[c]	90	39	16	24
0-I	119	38	15	25
0-II	116	38	18	25

[a] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > p > 0.01$ b = $0.01 > p > 0.001$ c = $p < 0.001$ - = not significant

[b] Medians were not presented if the total number of a particular tumor was three or less.

[c] Only organs with gross lesions were histologically examined from this exposure level at the 6-, 12-, and 18-month sacrifice intervals.

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-24. ETHYLENE OXIDE 2-YEAR VAPOR INHALATION STUDY: 24-MONTH FINAL SACRIFICE FREQUENCY OF EXPOSURE-RELATED NEOPLASMS FOR 110- TO 116-WEEK-OLD FISCHER 344 RATS

	ppm of Ethylene Oxide				
Organs/Findings/Sex	100[a]	33[a]	10[a]	Control I	Control II
Total number examined grossly					
Male	30	39	51	48	49
Female	26	48	54	60	56
Pituitary Adenomas					
Male	12/29[b]	13/39	15/51	16/48	13/49
Pancreas[c] Adenomas					
Male	5/30	1/2	2/3	2/48	5/49
Subcutis[d] Fibromas					
Male	10/28(c,c,c)	1/34	8/48(a,a,b)	1/44	2/47
Peritoneum Mesotheliomas					
Male	4/30	4/39	2/51	1/48	1/49
Spleen Mononuclear cell leukemias					
Male	8/30	10/39	9/51	5/48	8/49
Female	15/26(c,c,c)	14/48(b,b,b)	11/54(-,-,a)	5/60	6/55

[a] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

$a = 0.05 > p > 0.01$ $b = 0.01 > p > 0.001$ $c = p < 0.001$ - = not significant

[b] Numerator equals number of rats with specified finding. Denominator equals number of rats for which specified tissues were examined.

[c] Tissues from 33- and 10-ppm groups examined only if gross lesions were present. Since tissues were not examined from all rats, data from the 33- and 10-ppm groups were not statistically compared with data from other groups.

[d] Examined only if gross lesions were present (except flank region skin and subcutis, which was routinely examined microscopically).

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-25. ETHYLENE OXIDE 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF EXPOSURE-RELATED NEOPLASMS AT 24-MONTH FINAL SACRIFICE AND IN FISCHER 344 RATS DYING SPONTANEOUSLY OR EUTHANIZED WHEN MORIBUND[a]

	ppm of Ethylene Oxide				
Organs/Findings/Sex	100[b]	33[b]	10[b]	Control I	Control II
Pituitary Adenomas Male	24/79[c]	16/79	26/79	24/79	19/78
Pancreas[d] Adenomas Male	11/80$^{(b,-,a)}$	1/43	2/32	2/80	5/80
Subcutis[e] Fibromas Male	15/78$^{(c,b,c)}$	3/75	10/77$^{(b,a,b)}$	1/76	3/78
Peritoneum Mesotheliomas Male	21/80$^{(c,c,c)}$	6/80$^{(-,-,a)}$	3/80	1/80	2/80
Spleen Mononuclear cell leukemias Male	25/80	23/80	21/80	20/80	18/80
Female	27/80$^{(c,a,c)}$	24/80$^{(b,a,b)}$	14/80	9/80	13/76

---

[a] Concerning the animals that died spontaneously or were euthanized when moribund, it was not specified whether tissues were examined microscopically only when gross lesions were present, or if all tissues were reviewed in this way. It is therefore assumed that all of the tissues from these animals were studied histologically, whether or not gross lesions were observed. Not to have performed such studies would have yielded erroneously low frequencies of exposure-related neoplasms.

[b] Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > p > 0.01$ b = $0.01 > p > 0.001$ c = $p < 0.001$ - = not significant

[c] Numerator equals number of rats with specified finding. Denominator equals number of rats for which specified tissues were examined.

[d] Tissues from 33- and 10-ppm groups were examined only if gross lesions were present. Since tissues were not examined from all rats, data from the 33- and 10-ppm groups were not statistically compared with data from other groups.

[e] Examined only if gross lesions were present (except flank region skin and subcutis, which was routinely examined microscopically).

SOURCE: Adapted from Snellings et al., 1981.

number of treated animals that died spontaneously or were euthanized when moribund. When the tumor incidence in this latter group was added to that for animals sacrificed at 24 months, the numbers were much higher than controls and were statistically significant for the high-dose group versus controls (21/80 at 100 ppm, 6/80 at 33 ppm, 3/80 at 10 ppm vs. 1/80 for the Control I group and 2/80 for the Control II group) (Table 9-25).

Pancreatic adenomas were statistically significant for the male high-dose group sacrificed at 24 months and the animals that died spontaneously or were euthanized when moribund (11/80 at 100 ppm, 1/43 at 33 ppm, 2/32 at 10 ppm vs. 2/80 in the Control I group and 5/80 in the Control II group) (Table 9-25). Tissues from the 33 and 10 ppm groups were examined only if gross lesions were present in the 24-month sacrifice group, which may explain the paucity of tumors in these groups (Table 9-24). The denominator in Table 9-25, the number of rats for which the specified tissue was examined, may be erroneously high for the data combining the 24-month sacrifice with the animals that died spontaneously or were euthanized when moribund.

While Tables 9-23 and 9-24 show no significant increase in the frequency of pituitary adenomas in the groups of treated males, Table 9-23 shows some indication of a decreased time-to-tumor. In males, the first pituitary adenomas appeared at 15 months in the 100 and 33 ppm groups, and in the 17th or 18th month in all other groups; in females, the corresponding times were 10 months for the 100 ppm group versus at least 15 months for all other groups. The time-to-tumor decreased significantly with increasing dose ($p < 0.01$ for males, $p < 0.0001$ for females), suggesting that the normal incidence of pituitary adenomas was accelerated by exposure to ethylene oxide.

An increased frequency of mononuclear cell leukemia was observed in the ethylene oxide-treated animals at the 24-month sacrifice interval (Table 9-24).

Statistical significance was observed in females in both the 100 and 33 ppm groups versus combined controls ($p < 0.01$). The responses for the 24-month sacrifice were 15/26 (58%), 14/48 (29%), and 11/115 (10%) for the 100, 33, and 10 ppm groups and combined controls, respectively. The frequencies for male rats were not significantly increased in the treated versus the control groups.

In females, the results for animals dying spontaneously or euthanized when moribund and for those sacrificed at 24 months remained statistically significant for the two higher-dose groups versus combined controls. The frequencies for females (Table 9-25) were 27/80 (34%), 24/80 (30%), 14/80 (18%), and 22/156 (14%) for the 100, 33, and 10 ppm groups and combined controls, respectively, with statistically significant differences in the two higher-dose groups versus combined controls ($p < 0.01$) and a significantly positive linear dose-response trend ($p < 0.01$). The trend became even stronger ($p < 0.00001$) when the proportions were adjusted for early mortality. These data suggest that exposure to ethylene oxide not only increased the total incidence of leukemia but also accelerated its rate of development (Figure 9-3). The authors also reported that the number of female rats with three or more tumors was significantly ($p < 0.001$) increased in the 100 ppm group as compared to the controls.

A letter to the U.S. Environmental Protection Agency (Browning, 1982) stated that a recent histologic examination of all brain tissue from the Snellings et al. (1981) study revealed the presence of primary brain neoplasms (see Tables 9-26 and 9-27, and Table 9-33 in Section 9.5.3.3.2). These tumors were shown to be statistically significant by the Fisher Exact Test in both males and females.

In summary, ethylene oxide has produced significant increases of several tumor types in rats. A dose-related increase in mononuclear cell leukemia occurred in female rats. The occurrence of pituitary adenoma appeared to be

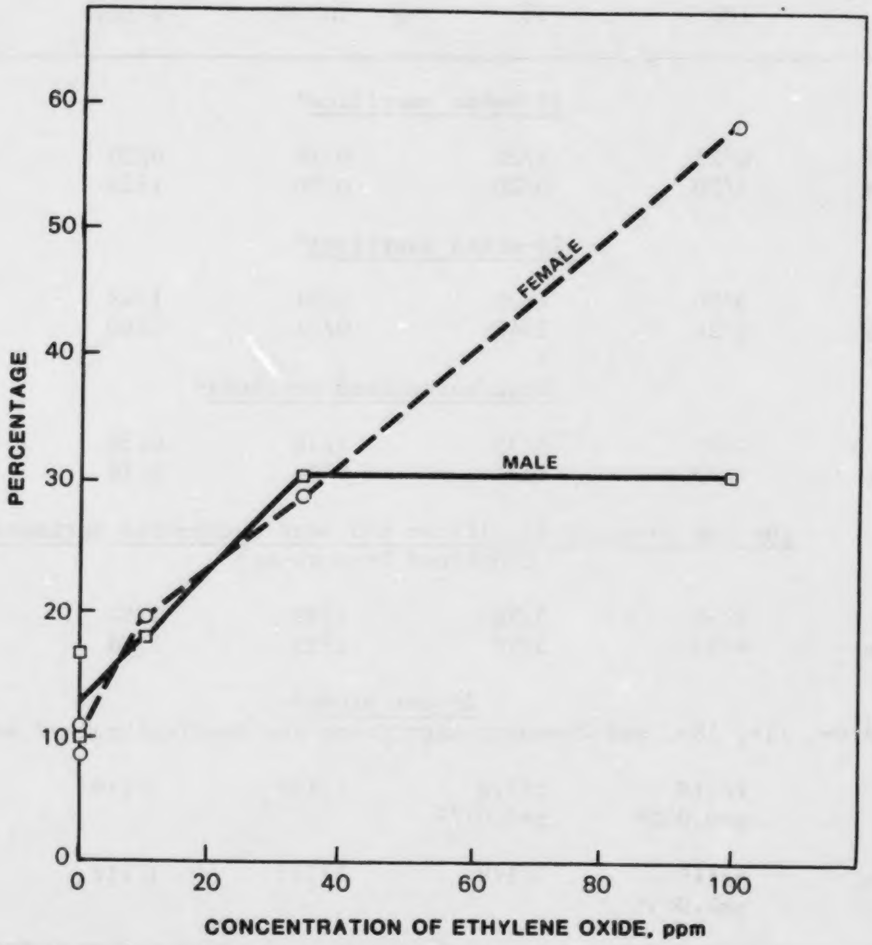


Figure 9-3. Percentages of male and female Fischer 344 rats with histologically confirmed mononuclear cell leukemia at 24-month sacrifice.

Source: Snellings et al. (1981).

TABLE 9-26. ETHYLENE OXIDE 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF PRIMARY BRAIN NEOPLASMS IN FISCHER 344 RATS

	Exposure level (ppm)				
Sex	100	33	10	0 (CI)	0 (CII)
		18-month sacrifice[a]			
Male	0/20	1/20	0/20	0/20	0/20
Female	1/20	0/20	0/20	1/20	0/20
		24-month sacrifice[a]			
Male	3/30	1/39	0/51	1/48	0/49
Female	2/26	2/48	0/51	0/60	0/56
		Dead/euthanized moribund[a]			
Male	4/49	3/39	1/28	0/30	0/29
Female	1/53	1/31	1/24	0/18	0/20
		18- and 24-month sacrifices and dead/euthanized moribund[a] (Combined from above)			
Male	7/99	5/98	1/99	1/98	0/98
Female	4/99	3/99	1/95	1/98	0/96
		2-year study[b] (Combined 6-, 12-, 18-, and 24-month sacrifices and dead/euthanized moribund animals)			
Male	7/119 p=0.002[c]	5/118 p=0.017[c]	1/119	1/118	0/118
Female	4/119 p=0.045[c]	3/119	1/115	1/118	0/116

---

[a] Numerator equals the number of brains with primary neoplasms. Denominator equals total number of brains examined microscopically.

[b] Numerator equals the number of brains with neoplasms. Denominator equals total number of brains examined microscopically. Although animals sacrificed at 6 and 12 months are included, no brain neoplasms were discovered in these groups. The 6- and 12-month animals can be eliminated by subtracting 20 from each denominator.

[c] Fisher Exact Test.

SOURCE: Adapted from Snellings et al., 1981.

TABLE 9-27. ETO 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF PRIMARY BRAIN NEOPLASM TYPES IN FISCHER 344 RATS

(Combined data for 6-, 12-, 18-, and 24-month sacrifices, and dead/euthanized moribund animals)

	Exposure level (ppm)				
Neoplasm type	100	33	10	0 (CI)	0 (CII)
	Males[a]				
Granular cell tumor	1/119	1/118	1/119	0/118	0/118
Astrocytoma/oligodendroglioma/mixed glioma	5/119	2/118	0/119	1/118	0/118
Malignant reticulosis-microglioma	1/119	2/118	0/119	0/118	0/118
	Females[a]				
Granular cell tumor	1/119	1/119	0/118	1/118	0/116
Astrocytoma/oligodendroglioma/mixed glioma	2/119	2/119	1/118	0/118	0/116
Malignant reticulosis-microglioma	1/119	0/119	0/118	0/118	0/116

[a] Numerator equals the number of brains with primary neoplasms. Denominator equals total number of brains examined microscopically. Although animals sacrificed at 6 and 12 months are included, no brain neoplasms were discovered in these groups. The 6- and 12-month animals can be eliminated by subtracting 20 from each denominator.

SOURCE: Adapted from Snellings et al., 1981.

accelerated in female rats exposed to 100 ppm, although there was no statistically increased incidence of these tumors. The frequency of peritoneal mesothelioma was treatment-related in the male rats exposed to 100 and 33 ppm. Further, a significant increase occurred in subcutaneous fibromas in male rats. Increases in brain neoplasms were also observed in both sexes.

9.5.1.2.2. Lynch et al. (1982) Inhalation Study (NIOSH) -- Another chronic inhalation study (unpublished draft) on ethylene oxide and propylene oxide was performed by the National Institute for Occupational Safety and Health (NIOSH) (Lynch et al., 1982). In the present report, only the preliminary findings of the ethylene oxide section of the study will be discussed. Male Fischer 344 rats (80 in each group) and 12 male cynomolgus monkeys were exposed to ethylene oxide at either 50 or 100 ppm for 7 hours/day, 5 days/week, for 24 months. Each treatment group consisted of 80 rats and 12 monkeys at the start of the study. Rats and monkeys were housed together in the same chambers during the 7-hour exposure period. Food and water were available ad libitum except during the exposure periods. In analyzing for carcinogenicity, only limited data were available for monkeys because of their longer lifespans; however, the authors reported that there was no evidence of leukemia in any of the exposed monkeys.

An overall statistically significant depression in weight gain was noted for ethylene oxide-exposed rats. This development, which appeared to begin at about week 7 for the 100 ppm group and at week 15 for the 50 ppm group, continued throughout the study. Survival was also adversely affected by exposure to ethylene oxide, with estimated mean survival times of greater than 720 days for the controls, 690 days for the 50 ppm group, and 653 days for the 100 ppm group. An outbreak of mycoplasma infection also caused an abrupt decline in survival at about 480 days into the study.

With respect to pathology, the authors reported that the livers and spleens of the ethylene oxide-exposed rats were the only organs for which histopathologic evaluations were completed. While the results are preliminary (Table 9-28), the data obtained at terminal sacrifice indicate that the incidence of leukemia followed a dose-response pattern ranging from 33.3% in controls to 64.3% in the 100 ppm group ($p = 0.07$, Table 9-28). The one-tailed test for linear trend at terminal sacrifice was significant at the $p < 0.05$ level. Using a two-tailed test, the significance level was $p = 0.08$. These preliminary data, therefore, do provide some evidence of ethylene oxide-induced leukemia. The data from moribund sacrifice and deaths (Table 9-28) merely accentuate both the early toxicity and the mortality in the 100 ppm group as compared with the other groups, and the relatively high leukemia rates in these rats. Neither these rats nor the total was significantly higher than controls.

TABLE 9-28. LEUKEMIA INCIDENCE IN MALE FISCHER 344 RATS EXPOSED TO ETHYLENE OXIDE FOR 2 YEARS[a]

	Leukemia incidence		
Treatment group	Terminal sacrifice only (%)	Moribund sacrifice and death (%)	Terminal sacrifice plus moribund sacrifice and death (total)
Control	7/21 (33.3%)	5/18 (27.9%)	12/39 (30.8%)
Ethylene oxide, 50 ppm	12/27 (44.4%)	26/52 (50.0%)	38/79 (48.1%)
Ethylene oxide, 100 ppm	9/14 (64.3%)[b]	21/62 (33.9%)	30/76 (39.5%)

[a] Based on histopathologic evaluation of spleens.
[b] $p = 0.07$ based on the one-tailed Fisher Exact Test.

SOURCE: Lynch et al., 1982.

Lynch et al. (1982) also reported that exposure to ethylene oxide significantly increased the incidence of peritoneal mesotheliomas. These tumors were present on the tunica vaginalis surrounding the testes and epididymis, and occasionally spread to the peritoneal cavity. A nonsignificant increase in pheochromocytomas was observed in exposed groups (Table 9-29).

Lynch et al. (1982) reported the following incidences of mixed-cell gliomas in male rats: 0/76 in controls, 2/77 in the 50 ppm group, and 5/79 in the 100 ppm group. The term "glioma" was used because the tumors contained both astrocyte and oligodendroglia cells within the tumor. These findings are significant because the above-described tumors are unusual in Fischer 344 rats. Additional data collected from this study are currently being evaluated, and a final comprehensive report is scheduled to be published within a year.

9.5.1.2.3. Summary of Animal Studies -- The Snellings et al. (1981) study, which showed an increase in leukemia in Fischer 344 rats, is also supported by a preliminary NIOSH study (Lynch et al., 1982, Table 9-28) in which an increase in leukemia appeared in rats of the same strain but of a different sex and with mycoplasma instead of SDA viral infections. Increases in peritoneal mesotheliomas were observed in both studies (Snellings et al., 1981 and Lynch et al., 1982), and significant increases in subcutaneous fibromas in the males were observed in the Snellings study. Snellings et al. (1981) also concluded that the frequencies among female rats with more than two neoplasms were significantly greater for all three groups when compared to combined controls.

Further, both studies found significant increases in brain neoplasms, a development that requires further review in terms of its possible value for risk evaluation. Like the finding of gliomas in male rats reported previously, these studies are significant because brain neoplasms are unusual in the Fischer 344 strain of rats.

In 1980, the National Toxicology Program (NTP) began a cancer bioassay in B6C3F1 mice (inhalation exposure). Exposure to ethylene oxide at 0, 5, and 100 ppm for 6 hours/day, 5 days/week began in August 1981. The final report is expected in mid-1985.

TABLE 9-29. INCIDENCE OF NEOPLASTIC LESIONS IN MALE FISCHER 344 RATS EXPOSED TO ETHYLENE OXIDE FOR 2 YEARS[a]

Organs/Findings	Exposure level (ppm) Control	 50	 100
Adrenal			
Pheochromocytomas	8/78	14/77	13/78
Brain			
Gliomas (mixed-cell)	0/76	2/77	5/79 ($p = 0.032$)[b]
Body cavity			
Peritoneal mesotheliomas	3/78	9/79	21/79 ($p = 4.95 \times 10^{-5}$)[b]
Spleen			
Mononuclear cell leukemia	24/77	38/79 ($p = 0.22$)[b]	30/76

[a] Each group consisted of 80 male rats. Denominators of less than 80 reflect tissues accidentally lost or tissues that could not be examined histologically due to autolysis.

[b] Fisher Exact Test.

SOURCE: Lynch et al., 1982.

9.5.2. Epidemiologic Studies

9.5.2.1. JOYNER (1964) -- Joyner (1964) conducted a health evaluation of employees at an ethylene oxide plant in Texas. The evaluation included a physical examination of 37 male ethylene oxide operators, aged 29 to 56, and a similar number of age-matched controls. The operators were reported to have been exposed to ethylene oxide at approximately 5 to 10 ppm for the durations of their service. The controls, who were chosen from operators assigned to other production units, had been exposed to many different agents encountered in the petrochemical industry. The author stated that the mean length of service for the control group was 11 2/3 years, as compared with 10 2/3 years for the exposed group. The author used company medical records for the period 1952-1963 to compare the exposed group and controls with respect to days lost for illness, specific diagnoses, and initial visits for respiratory, gastrointestinal, or genitourinary complaints. The author found that the ethylene oxide operators who were currently employed exhibited less absenteeism, fewer symptoms, and fewer diagnosed illnesses (including malignant neoplasms) than the controls.

The author also reviewed the medical records of nine operators who had experienced accidental exposures in the previous 10 years, and seven workers other than operators who had experienced accidental exposures in the previous 8 years. Twelve of the accidental exposures were reported to be dermal exposures, while three were reported to be inhalation exposures; one exposure was reportedly to "vapor." Most of the dermal exposures produced burns. The vapor exposure produced conjunctivitis. Two of the persons with inhalation exposure suffered no symptoms; the third developed nausea and vomiting, which lasted several hours. The authors reported that the persons identified as having had accidental exposures did not exhibit any recurring medical problems. The one person who had suffered symptoms from the inhalation exposure was no longer with the company and could not be traced.

Additionally, the author reviewed the medical records of eight persons who had previously worked as ethylene oxide operators for 100 months or more but who had since been transferred to another division. Among persons formerly employed as ethylene oxide operators for 100 months or more, no significant differences were found in the incidence of illness, symptoms, complaints, or absenteeism when compared to the study cohort or to controls; very few data were presented in this regard, however.

This study is inadequate for use in evaluating the carcinogenicity of ethylene oxide for several reasons. First, it is primarily a cross-sectional study of ethylene oxide operators who were employed as such at the time of the study. Workers who had developed cancer would probably no longer have been employed at the plant. Secondly, the period of observation, which in this study is the same as the duration of exposure for the current operators, may have been too short to allow adequate assessment of a carcinogenic effect. Cancer latency may be as long as 20 to 30 years; the longest observation period among current operators in this study was 16 1/3 years. The mean exposure for current operators was 10 2/3 years. For those with accidental exposures, the longest follow-up was 10 years. For the eight workers with over 100 months (8 1/3 years) of exposure, the length of follow-up was not indicated. Third, the sample sizes studied were so small that only an extremely large carcinogenic effect could be detected.

9.5.2.2. EHRENBERG AND HALLSTROM (1967) -- Ehrenberg and Hallstrom (1967) conducted a hematologic investigation of workers at a factory that manufactured and used ethylene oxide. A preliminary investigation in 1960 revealed certain hematologic differences between 28 exposed persons who worked in an area of the factory "where leakage of ethylene oxide from tube joints, pumps, etc. was possible (and at least occasionally occurred)," and 26 controls in other departments not working in contact with ethylene oxide. The sex of the study subjects was not reported. The ages of persons in the exposed group were reported to be about the same as those in the control group. The exposed persons were reported to have been active in the ethylene oxide department for 2 to 20 years, with an average of 15 years. One case of leukemia (chronic lymphatic type) was observed in the exposed group; the expected number of leukemia cases in the exposed group was not reported. No cases of leukemia were found in the controls. Three cases of anisocytosis were found in the exposed group and none in the controls, a finding which the authors suggested may indicate a disturbed bone marrow function. Hemoglobin values were reported to be significantly ($p < 0.05$) lower in the exposed group than in the controls, and lymphocytes per mm^3 were reported to be significantly ($p < 0.01$) higher in the 27 exposed healthy persons than in the 20 healthy controls (the presence of disease may affect the white blood cell count; thus, only "healthy" persons were considered in the latter comparison). It should be noted that three persons who were reported to have been accidentally exposed to high levels of ethylene oxide were added to the exposed group for the lymphocyte/mm^3 comparison (for a total of 31 persons in the exposed group). The authors did not state where these three persons worked or even whether they worked in the factory.

Because of these differences relating to hemoglobin and lymphocytes, and because ventilation was improved in the plant, the authors did a second study of the factory workers in 1961. The second study was expanded to include all of the workers in the plant. Workers were divided into four categories: "66 persons not working with ethylene oxide (including the 1960 control group); 86 persons intermittently working in ethylene oxide premises; 54 persons who had once been working in contact with ethylene oxide for some period of time; and 37 persons permanently working in the ethylene oxide area (including the 1960 exposed group)." The only hematologic analysis in the second study was for lymphocytes. The authors found an elevated lymphocyte count in the exposed group as compared with controls, but this difference was not significant ($p > 0.05$) for either healthy individuals or the total group. The authors suggested that this lack of a significant difference could possibly be attributed to improved ventilation and safety control in the factory, the small number (17) of healthy persons in the group permanently exposed (versus 27 healthy exposed individuals in the 1960 investigation), and/or the average age difference between the exposed and control groups. The average age of the enlarged control group was reported to be "significantly" lower than that of the exposed group, and, in general, a decrease in lymphocyte count with age was found. A significant age difference between the exposed group and the controls was not present in the 1960 examination. It should be noted that for those persons examined in the 1960 investigation, a significant difference in average lymphocyte count between the exposed group and the controls occurred again when the two groups were examined in 1961.

The authors also compared the number of chromosome aberrations in eight persons accidentally exposed to ethylene oxide with that in a control group of 10 persons, and found that chromosome aberrations were significantly elevated in the exposed group. Details of the statistical analysis were not given.

In conclusion, Ehrenberg and Hallstrom (1967) found one leukemia case among 28 workers exposed to ethylene oxide. The authors indicated that the probability of such an occurrence was small, but its statistical significance was not calculated. The results of the study also suggest that ethylene oxide may elevate lymphocyte counts and reduce hemoglobin values.

9.5.2.3. HOGSTEDT ET AL. (1979a, 1984) -- A follow-up study for the years 1961 through 1977 of these same workers with regard to mortality and cancer incidence was done by Hogstedt et al. (1979a). A subsequent study by Hogstedt et al. (1984) followed the mortality of the cohort for the years 1978 through 1982 and the incidence for the years 1978 through 1981. A period of at least 10 years of follow-up from date of first employment was required in order for a member of the cohort to be considered at risk. The authors reported that the workers in this factory were exposed to various chemicals. During the period from 1941 to 1947, it was estimated that the air concentrations were 5 mg/m^3 ethylene chlorohydrin, 100 mg/m^3 ethylene dichloride, 0.05 mg/m^3 bis(2-chloroethyl) ether, and 600 mg/m^3 ethylene. The authors also cited the possibility that concentrations up to 1,199 times greater than those reported may have occurred for short periods of time. For ethylene oxide, the exposure was reported to be probably < 25 mg/m^3, although there were occasional exposures to the chemical at 1300 mg/m^3 (odor threshold). During the 1950s and until 1963, the authors reported that the average air concentration of ethylene oxide in the factory was probably 10 to 50 mg/m^3, although peaks above the odor threshold still occurred. Random samples in the 1970s showed a range of 1 to 10 mg/m^3 for ethylene oxide and 10 to 25 mg/m^3 for propylene oxide, with the latter concentrations occasionally being as high as 120 to 150 mg/m^3.

Hogstedt et al. (1979a) reported that the study included three subcohorts: 66 men who had never taken part in work involving exposure to ethylene oxide, 86 "intermittently" exposed men (maintenance workers), and 89 men whose work involved full-time exposure. The same number of men were reported by Hogstedt et al. (1984) for the non-exposed and full-time exposed groups, but they reported only 79 men in the "intermittently" exposed group. No explanation for this difference was provided by the authors.

By the end of the second follow-up of these workers (Hogstedt et al., 1984), a total of 12 cancer deaths had been observed in the full-time exposed group, while only 4.8 were expected. The Carcinogen Assessment Group (CAG) calculated that the probability of this occurring was less than 0.01. There were no statistically significant differences between the observed and expected number of cancer deaths in the other two exposure groups. Seven of the 12 cancer deaths seen in the fulltime exposed cohort were either from cancer of the stomach (5 deaths) or from leukemia (2 deaths). Deaths from both causes were significantly elevated in comparison with the numbers expected (5 observed versus 0.6 expected for stomach cancer, $p < 0.01$, as calculated by the CAG; and 2 observed versus 0.15 expected for leukemia deaths, $p < 0.05$, as calculated by the CAG). One of the leukemia deaths was from chronic lymphatic leukemia, and the other was from acute myeloid leukemia. The death from chronic lymphatic leukemia may well have been the same case that was reported in the Ehrenberg and Hallstrom (1967) study. Excess mortality for cerebrovascular disease during the period 1961 through 1982 was also statistically significant (5 observed, 1.5 expected; $p < 0.05$, as calculated by the CAG) among the full-time operators. Although the maintenance group showed no overall excess cancer mortality, the cancer deaths that occurred in this group were restricted to cancers of the esophagus and stomach and to leukemia. The leukemia death was from chronic lymphatic leukemia.

Hogstedt et al. (1984) examined the observed and expected numbers of deaths from various causes, including different cancer sites, by 1-4, 5-9, and 10+ years of exposure. No response by length-of-exposure was found for any of the causes of death, but the data were rather limited for this type of analysis.

Cases of cancer in the study group were determined using the Swedish Cancer Registry. No indication of the completeness of ascertainment of the Registry was given. Seventeen cases versus 7.9 expected ($p < 0.01$) were identified among full-time exposed workers for the period 1961 through 1981. These included three cases of leukemia versus 0.24 expected ($p < 0.01$, as calculated by the CAG). Included in the three leukemia cases were the individual with acute myeloid leukemia and the individual with chronic lymphatic leukemia, both of whom had died, as well as an individual with chronic myeloid leukemia. One case of stomach cancer was reported in addition to the five cases in which individuals had died. The expected number of stomach cancers was not indicated.

In summary, deaths from cancer of all sites, deaths from stomach cancer, and deaths from leukemia were each significantly ($p < 0.05$) elevated among the full-time exposed cohort. The total number of malignancies and the number of leukemia cases were also significantly ($p < 0.01$) elevated in this group. Workers in the full-time exposed cohort were exposed to several chemical agents, however, and the excess cancer incidence and mortality in this cohort cannot necessarily be ascribed to the ethylene oxide exposure.

9.5.2.4. HOGSTEDT ET AL. (1979b, 1984) -- Hogstedt et al. (1979b, 1984) reported on the morbidity and mortality of a group of sterilizing operators exposed to 50% ethylene oxide and 50% methyl formate. The 1979b report indicated that only seven persons worked with the sterilization process. However, treated boxes (supposedly containing the sterilized equipment) were stored in a hall where 30 women worked, and because of leakage from the boxes, the average exposure in the storage hall was reportedly higher than in the sterilization room. Exposure measurements made in 1977 showed storage hall concentrations of 2 to 70 ppm with 8-hour time-weighted average concentrations being calculated at 20 ± 10 ppm. The concentration was 1,500 ppm inside newly sterilized boxes and 150 ppm on the floor outside the boxes.

During the period from 1968 to 1977, 70 persons had been employed at some time in the storage hall, and another 160 had been employed in the neighboring rooms or as sterilizing operators. Of these, 69 (63 women, 6 men) and 134 (90 women, 44 men) had worked for a year or more, respectively (Hogstedt et al., 1984). In the Hogstedt et al. (1979b) report, the authors indicated that among this group of workers three cases of leukemia had occurred. One of these reported cases was actually a Waldenström's macroglobulinemia, however, and it was subsequently reported (Hogstedt et al., 1984) that this case should have been classified as a non-Hodgkin's lymphoma. However, according to the Eighth Revision of the International Classification of Diseases (ICD), which is the revision used in the Hogstedt et al. (1984) report, Waldenström's macroglobulinemia is classified as a plasma protein abnormality and not as a neoplasm.

The two leukemia cases were among women who worked in the storage hall. One of the cases was a woman who began working in the storage hall in 1966, was diagnosed with chronic myeloid leukemia in early 1972 at the age of 51, and died in 1977. The other case was a woman who began working in the storage hall in 1968, was diagnosed with acute myelogenetic leukemia in early 1977 at the age of 37, and as of July 1978 was reported to be in complete remission. Hogstedt et al. (1984), however, reported that she subsequently died during the extended follow-up period (1978-1982). A third leukemia death was reported by Hogstedt et al. (1984). This case was a woman who had had intermittent exposure, passing through the storage hall 2 to 4 times per day. She was employed during 1969-1972, and in 1979 was diagnosed with blast leukemia and died the same year.

Two males were reported to have died from cancer in the period 1968-1982. One was the case of Waldenström's macroglobulinemia mentioned earlier. As previously indicated, this death would not be considered a cancer death by the Eighth Revision of the ICD. The other death from cancer was not specified by site. Observed and expected cancer mortality, total mortality, and mortality from cancer of the lymphatic and hematopoietic systems for both the earlier (Hogstedt et al., 1979b) and later (Hogstedt et al., 1984) observation periods as well as the total observation period is reported in Table 9-30.

With regard to morbidity, ten cases of cancer among the female sterilizer workers had been reported to the Cancer Registry versus 5.2 expected ($p < 0.05$, as calculated by the CAG) during the period 1961-1981; there were three cases of cancer among male sterilizer workers (excluding the case of Waldenström's macroglobulinemia) versus 1.8 expected. The excess morbidity among females was mainly due to the three cases of leukemia (0.1 expected, $p < 0.01$, as calculated by the CAG) and two cases of malignant cancer of the cervix (0.4 expected). The cases among men were due to tumors of the stomach, colon, and rectum.

Hogstedt et al. (1979b) suggested that the combination of ethylene oxide and methyl formate may produce a special carcinogenic risk, since methyl formate, the authors indicated, exhibits its antibacterial effect by affecting DNA structure. No literature reference was cited by the authors as to this point, however. A literature search conducted for the Carcinogen Assessment Group by the Environmental Mutagen Information Center at the Oak Ridge National Laboratory (Francis, 1985) failed to find any literature citations for mutagenicity studies of methyl formate.

TABLE 9-30. OBSERVED AND EXPECTED NUMBER OF DECEASED AMONG 153 WOMEN AND 50 MEN WITH CONTINUOUS OR INTERMITTENT EXPOSURE TO ETHYLENE OXIDE[a]

		Women				Men				Women + Men	
	Causes	1968-77		1978-82		1968-77		1978-82		1968-82	
ICD[b]	of death	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
1-999	Total	2	2.9	3	2.7	4	2.6	2	2.2	11	10.4
140-209	All tumors	2	1.2	3	1.1	0	0.6	1	0.5	6	3.4
200-207	Lymphatic and hemato-poietic tissue	1	0.1	2	0.08[c]	0	0.06	0	0.05	3	0.3[c]

[a] The observed case of Waldenström's macroglobulinemia has been deleted from this table. It had appeared in the table by Hogstedt et al. (1984).

[b] International Classification of Diseases, Eighth Revision.

[c] $p < 0.01$, calculated by the CAG.

SOURCE: Adapted from Hogstedt et al., 1984.

9.5.2.5. MORGAN ET AL. (1981) -- Morgan et al. (1981) conducted a retrospective study of 767 workers potentially exposed to ethylene oxide who had worked for at least 5 years at a Texaco Chemical Company plant in Port Neches, Texas, between January 1955 and December 31, 1977. The authors provided no analysis of the cohort with respect to length of follow-up. An industrial survey of the plant (performed in July 1977) showed that the 8-hour time-weighted average exposure to ethylene oxide was "well below" 50 ppm, except in the area around the tank car loading operations, where readings were as high as 6,000 ppm. Among the 767 male workers potentially exposed to ethylene oxide in the study cohort, there were 11 deaths from malignant neoplasms, where 15.24 would have been expected on the basis of U.S. vital statistics.

There were more deaths than expected from pancreatic cancer (SMR* = 377, 3 observed versus 0.8 expected), bladder cancer (SMR = 322, 1 observed versus 0.31 expected), brain and central nervous system cancer (SMR = 285, 2 observed versus 0.7 expected), and Hodgkin's disease (SMR = 570, 2 observed versus 0.35 expected). Although the 95% lower confidence limits for these SMRs were all less than 100, the number of deaths from pancreatic cancer and the number of deaths from Hodgkin's disease are each significantly ($p < 0.05$) more than expected by hypothesis testing using the Poisson test. Excess mortality from leukemia was not found. Because their study cohort was small and because excess cases of leukemia following exposure to ethylene oxide were found in the studies by Hogstedt et al. (1979a, b), the authors calculated the magnitude of the relative risk of mortality from leukemia, given the sample size of the cohort, that could be detected at the 95% confidence level with a power of 80%. This relative risk was calculated to be 10.5 (SMR of 1050). In conclusion, it should be stated that the observed mortalities from pancreatic cancer and from Hodgkin's disease were each significantly elevated among the study cohort, and that the study cohort may have been too small for an adequate evaluation of the risk of mortality from leukemia or other cancer types. Furthermore, there was no indication by the authors that sufficient allowance had been made for a cancer latency period.

---

*Standardized mortality ratio.

9.5.2.6. THIESS ET AL. (1982) -- Thiess et al. (1982) conducted a cohort mortality study of 602 persons who had been employed for 6 months or longer in the alkylene oxide (ethylene oxide/propylene oxide) production or processing areas of nine BASF Aktiengesellschaft, Ludwigshafen plants in West Germany during the period from 1928 to 1980. Vital status was ascertained for 523 of the 536 German employees in the cohort, while that of only 30 of the 66 non-German employees could be determined. Thus, the percentage of overall follow-up in this study was 92% (553 of 602). In addition to alkylene oxides, the workers were reported to have been exposed to a variety of other compounds.

The expected mortality for the total cohort and for those within the cohort who were observed for a minimum of 10 years was calculated using mortality data for Ludwigshafen, Rhinehessia-Palatinate, and the Federal Republic of Germany. The observed and expected numbers of cancer deaths for those persons observed for at least 10 years are reported in Table 9-31. The observed number of deaths from cancer of any site was not significantly ($p < 0.05$) higher than that expected based on mortality data for Ludwigshafen, Rhinehessia-Palatinate, or the Federal Republic of Germany. Deaths from cancer of the brain among alkylene oxide workers who were followed for at least 10 years did approach statistical significance ($p < 0.07$), however, in comparison with those expected based on Ludwigshafen or Rhinehessia-Palatinate mortality data.

TABLE 9-31. COMPARISON OF OBSERVED NUMBERS OF CANCER DEATHS IN BASF-AKTIENGESELLSCHAFT, LUDWIGSHAFEN PLANTS 1928-80 FOR PERSONS HAVING 10 YEARS OF OBSERVATION FOLLOWING EXPOSURE TO ALKYLENE OXIDE WITH THAT EXPECTED BASED ON MORTALITY STATISTICS FOR RHINEHESSIA-PALATINATE 1970-75, LUDWIGSHAFEN 1970-75, AND THE FEDERAL REPUBLIC OF GERMANY 1971-74, BY ICD CODE AND CAUSE OF DEATH

			Rhinehessia-Palatinate 1970-75		Ludwigshafen 1970-75		Federal Republic of Germany 1971-74	
ICD No.[a]	Cause of death	Observed deaths	No.	P-value	No.	P-value	No.	P-value
151	Malignant tumor of the stomach	2	1.852	0.552	1.765	0.527	2.033	--[b]
156	Malignant tumor of the gall bladder	1	0.201	0.182	0.243	0.216	--[c]	--[c]
162	Malignant tumor of the bronchii	4	3.769	0.520	3.956	0.568	--[c]	--[c]
188	Malignant tumor of the urinary bladder	1	0.469	0.374	0.532	0.413	--[c]	--[c]
191	Malignant tumor of the brain	1	0.071	0.068	0.066	0.064	--[c]	--[c]
193-199	Squamous cell carcinoma of unknown primary site	1	0.743	0.525	1.047	--[c]	--[c]	--[c]
Total of malignant tumors in ICD 140-199[c]		10	--[d]	--[d]	--[d]	--[d]	11.816	--[d]
205	Myeloid leukemia	1	0.148	0.138	0.145	0.135	0.756	0.531
230-239	Tumor of unknown character	1	0.454	0.365	0.426	0.347	--[c]	--[c]

[a] International Classification of Diseases, Eighth Revision.

[b] The probability of observed deaths occurring by chance was not provided by the authors because the observed deaths were fewer than expected.

[c] The authors did not report the number of deaths that would be expected in the cohort based on Federal Republic of Germany mortality rates for individual tumor sites other than stomach and myeloid leukemia.

[d] The authors did not report the number of deaths from tumor sites, ICD 140-199, that would be expected based on Rhinehessia-Palatinate or Ludwigshafen mortality data.

SOURCE: Adapted from Thiess et al., 1982.

The authors also compared the observed number of cancer deaths with that expected, using an internal cohort of 1,662 styrene workers. The minimum observation period of 10 years required for the comparison in Table 9-31 was not used for this analysis. Thus, in Table 9-32, there were 14 total observed cancer deaths, as opposed to 12 observed deaths in Table 9-31. These results are reported in Table 9-32. The relative risk of death from cancer of all sites in the alkylene oxide cohort in comparison to what would be expected based on cancer mortality in the styrene cohort was 1.48. Assuming that the numbers of observed and expected deaths (14 and 9.44, respectively) are both Poisson variables, the difference between the two is not statistically significant ($p < 0.05$). In the 65- to 74-year-old age group, the relative risk was 2.78. If it is assumed that both the observed and expected deaths are Poisson variables, the difference between the two is statistically significant at $p < 0.05$. It should be noted that although the authors reported in tabular form that 10 cancer deaths had occurred in the 65- to 74-year-old age group, the text indicated that 11 had occurred--a difference that obviously would function to lower the probability of the number of cancer deaths that was observed. A major problem in evaluating this result, however, is that the workers in the alkylene oxide cohort were exposed to a variety of chemicals in addition to ethylene oxide, some of which are known or suspected carcinogens. The authors did not compare the alkylene oxide and styrene cohorts with regard to the number of deaths by individual tumor site.

The authors also analyzed the cancer deaths by length of exposure, and did not find a dose-response. However, they gave no indication that the mortality analysis by length of employment had been adjusted for length of follow-up.

In summary, this study is inconclusive as to whether persons exposed to ethylene oxide are at an excess risk of death from cancer. There was a signi-

TABLE 9-32. RELATIVE RISKS OF DEATH FROM CANCER IN THE ALKYLENE OXIDE COHORT AS COMPARED WITH THE STYRENE COHORT, BY AGE[a]

Age group	Observed deaths	Expected deaths	Relative risk
15-24	--	--	--
25-34	--	0.35	--
35-44	--	0.47	--
45-54	--	1.61	--
55-64	4	3.41	1.17
65-74	10	3.60	2.78
75-84	--	--	--
Total	14	9.44	1.48

[a] In this analysis, a minimum observation period of 10 years was not made a requirement.

SOURCE: Adapted from Thiess et al., 1982.

ficant excess number of cancer deaths in the 65- to 74-year-old age group in the alkylene oxide cohort, as compared to that expected based on the mortality data for a group of styrene workers. A fact that may have confounded this result is that the alkylene oxide workers were exposed to a variety of chemicals in addition to ethylene oxide, some of which are known or suspected carcinogens. Deaths from cancer of any particular site were not found to be significantly ($p < 0.05$) in excess when the expected numbers of deaths for those sites were derived using mortality data for Ludwigshafen or Rhinehessia-Palatinate. Two problems with this study are the small sample size and the fact that only a little more than half of the cohort was observed for 10 years or more. In regard to leukemia mortality, for which Hogstedt et al. (1979a, 1984) had found an association with ethylene oxide exposure, the authors found that for those persons who had had more than 10 years of exposure, one case of myeloid leukemia occurred where only about 0.15 would have been expected based on local mortality data, but this difference was not statistically significant ($p < 0.05$).

9.5.2.7. SCHNORR (1982) -- A proportionate mortality study by Schnorr (1982) of decedents who had been members of District 1199 of the National Hospital and Health Care Workers Union found that the proportionate mortality ratio (PMR) for neoplasms of lymphatic and hematopoietic tissue (ICD code 200-209, Eighth Revision), as well as for other types of tumors, was significantly elevated for certain job categories (e.g., "service" and "nursing") that included job titles of personnel exposed to ethylene oxide (e.g., hospital central service employees, registered nurses, licensed practical nurses, and nurse's aides). Such job categories were relatively broad in their inclusion of job titles, however, and the results of the study with regard to a possible association of cancer risk with ethylene oxide exposure must therefore be judged inconclusive.

9.5.2.8. STUDIES IN PROGRESS -- Several cohort or case-control studies testing the association of ethylene oxide exposure and the risk of cancer are currently in progress or about to begin. A cohort mortality study of approximately 1,200 workers who were engaged in ethylene oxide production during their work history in the chemical industry in the Kanawha Valley, West Virginia, is currently being conducted by the National Institute for Occupational Safety and Health (NIOSH) and the Union Carbide Corporation. Nested case-control studies within the cohort will be done for certain kinds of deaths (e.g., leukemia). The results of the cohort study will not be available until early 1986. The results of the case-control study will not be available until some time later (Rinsky, personal communication).

NIOSH and the Health Industry Manufacturing Association are currently conducting a cohort mortality study of approximately 10,000 persons, consisting primarily of medical equipment manufacturing personnel who use ethylene oxide as a sterilant. Some exposure information on this cohort is available, but only for 1978 onward. The results will not be available until at least 1987, and a published report is expected about a year later.

The U.S. Environmental Protection Agency funded a case-control study of 70 cases of cancer of the lymphatic and hematopoietic tissue and 140 controls in District 1199 of the National Hospital and Health Care Workers Union to determine if an association existed between such cancers and occupational exposure to ethylene oxide and/or other substances. The study, conducted by Dr. Jeanne Stellman of Columbia University, has been completed but has not yet been published as of the date of this writing. No association between the cases and exposure to ethylene oxide was reported to be found; however, some problems with regard to ascertainment of exposure did exist (Schnorr, personal communication).

9.5.2.9. SUMMARY OF EPIDEMIOLOGIC STUDIES -- Three epidemiologic studies of persons occupationally exposed to ethylene oxide demonstrated a significant association between ethylene oxide exposure and cancer incidence or mortality. A study by Hogstedt et al. (1979a, 1984) found significantly ($p < 0.05$) increased mortality for stomach cancer and leukemia and significantly ($p < 0.01$) increased incidences of cancer of all sites and of leukemia among ethylene oxide production workers. Hogstedt et al. (1979b, 1984) found significantly ($p < 0.05$) increased incidences of leukemia and cancer of all sites and significantly ($p < 0.01$) increased mortality from leukemia among workers exposed to ethylene oxide used as a sterilant. The study by Morgan et al. (1981) found increased mortality from pancreatic cancer and Hodgkin's disease that is statistically significant ($p < 0.05$) by hypothesis testing.

Excess mortality from leukemia in the Hogstedt et al. (1979a, 1984) study and excess incidences of leukemia in the Hogstedt et al. (1979b) study were not limited to any particular types of leukemia. Excess deaths from leukemia in the Hogstedt et al. (1979a) study included one case of acute myeloid leukemia and two cases of chronic lymphatic leukemia. Excess cases of leukemia in the Hogstedt et al. (1979b) study included one case of acute myeloid leukemia, one case of chronic myeloid leukemia, and one case of "blast" leukemia. The expected numbers of deaths or cases by type of leukemia were not calculated in either study.

It should be noted that in all three of the above-referenced epidemiologic studies, exposure of the cohort to other chemicals besides ethylene oxide was reported to have occurred or probably occurred. In the Hogstedt et al. (1979a) study, reports were made of exposure to several chemicals, of which two, ethylene dichloride and bis(2-chloroethyl)ether, are recognized carcinogens. In the Hogstedt et al. (1979b) study, ethylene oxide-exposed workers experienced concurrent exposure to methyl formate. In the Morgan et al. (1981) study, there was no mention of exposure to chemicals other than ethylene oxide, but the fact that the study was conducted at a chemical plant would suggest that exposure to other chemicals did occur.

9.5.3. Quantitative Estimation. This quantitative section deals with the incremental unit risk for ethylene oxide in air and the potency of ethylene oxide relative to other carcinogens that the CAG has evaluated. The incremental unit risk estimate for an air pollutant is defined as the increased life-time cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of $1 \mu g/m^3$ of the agent in the air they breathe. These calculations are done to estimate in quantitative terms the impact of the agent as a carcinogen. Incremental unit risk estimates are used for two purposes: 1) to compare the carcinogenic potencies of several agents with each other, and 2) to give a crude indication of the population risk that would be associated with air or water exposure to these agents, if the actual exposures were known. Hereinafter, the term "unit risk" will always refer to incremental unit risk.

In the sections that follow, the general assessment procedures used by the CAG are discussed. These include animal-to-human extrapolation modeling, data selection, calculation of human equivalent doses, extrapolation modeling from human epidemiologic studies, and interpretation of the resulting estimates. Following this discussion, the CAG's unit risk calculations and relative potency estimates are presented.

9.5.3.1. PROCEDURES FOR THE DETERMINATION OF UNIT RISK FROM ANIMAL DATA — In developing quantitative estimates of carcinogenic risks, one or both of two types of data are utilized: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, at incidences determined by an extrapolation model.

There is, however, no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time, the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear nonthreshold dose-reponse relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). There is also some evidence from animal experiments that is consistent with the linear nonthreshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large-scale ED_{01} study at the National Center for Toxicological Research, and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Based on the above evidence of low-dose linearity, and because very few compounds exhibit low-dose responses that are superlinear, the linear nonthreshold model has been adopted as the primary basis for risk extrapolation in the low-dose region of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk; i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. The multistage model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

9.5.3.1.1. Description of the Low-Dose Animal Extrapolation Model -- Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL83, developed by Howe (1983). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper limit, q_1^*, is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk, $P_t(d)$, is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose are deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i\,(1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever χ^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

9.5.3.1.2. Selection of Data -- For some chemicals, a number of studies in different animal species, strains, and sexes, each run at varying doses and routes of exposure, are available. In such cases, choices must be made as to which of several data sets are appropriate for use with the chosen model. The following are the procedures used by the CAG in evaluating these data for the purpose of risk estimation:

1. The data on tumor incidence are separated according to organ sites or tumor types. The dose and tumor incidence data set used in the model is the set in which tumor incidence is statistically significantly higher than in controls for at least one test dose level, and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the data set having the larger sample size is selected for calculating carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment.

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

9.5.3.1.3. Calculation of Human Equivalent Dosages from Animal Data -- In calculating human equivalent dosages, it is necessary to correct for differences in metabolism among species and for the variations in absorption factors involved in different routes of administration.

Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner:

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e) and

W = average weight of the experimental animal

The lifetime average exposure is then

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rw^{2/3}} = s \times W^{1/3}$$

where r is the absorption rate for ethylene oxide (assumed to be 1).

When exposure is via inhalation, as with ethylene oxide, dose calculations at experimental exposures of up to 100 ppm are performed under the assumption that the compound is a completely water-soluble gas absorbed proportionally to the amount of air breathed in. While the CAG has previously used an existing methodology to determine dose equivalency in such cases, for ethylene oxide the total body dose resulting from exposure of male Fischer 344 rats to air concentrations of 100 ppm for 6 hours has been measured as 20.24 mg/kg (Tyler and McKelvey, 1980). At 10 ppm exposures under similar conditions, the measured dose was 2.7 mg/kg. Since daily exposures in the Snellings et al. (1981) study included 10 ppm and 100 ppm, the human equivalent dosage for the above exposure is estimated as

$$d_h = 20.24 \times 5/7 \div (70/0.42)^{1/3} = 2.63 \text{ mg/kg/day for 100 ppm}$$

and

$$d_h = 2.7 \times 5/7 \div (70/0.42)^{1/3} = 0.35 \text{ mg/kg/day for 10 ppm}$$

where 0.42 kg is the average weight of the male rat in the Snellings et al. (1981) study, 70 kg is the average weight of the adult human, and 5/7 is the fraction of days exposed. By interpolation, the 33 ppm exposure is estimated as 0.94 mg/kg/day in human equivalent doses.

9.5.3.1.4. Calculation of the Unit Risk from Animal Studies -- The risk associated with d mg/kg$^{2/3}$/day is obtained from GLOBAL83, and for most cases of interest to risk assessment, the 95% upper-limit risk can be adequately approximated by $P(d) = 1 - \exp(-q_1^* d)$. A unit risk in units X is simply the risk corresponding to an exposure of $X = 1$. To estimate this value, it is simply necessary to find the number of mg/kg$^{2/3}$/day that corresponds to one unit of X, and substitute this number into the above relationship. For ethylene oxide, human equivalent doses will first be calculated and then fitted, together with the observed responses, to the linearized multistage model. An equivalent method of calculating unit risk would be to use mg/kg/day for the animal exposures and then to increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1, 2, \ldots, k$$

and use the mg/kg/day equivalents for the unit risk values. In the section of this document that presents unit risk calculations from animal data, the final q_1^* will always represent the upper-limit potency estimate for humans.

9.5.3.1.5. Interpretation of Quantitative Estimates -- Unit risk estimates based on animal bioassays are only approximate indications of absolute risk in populations exposed to known carcinogen concentrations. This is true for several reasons. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans as compared to animals based on the relationship of weight to surface area is virtually without experimental verification as regards carcinogenic response.

Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

Unit risk estimates can give rough indications of the relative potencies of given agents as compared with other carcinogens. Such comparisons are, of course, most reliable when based on studies in which the test species, strain, sex, and route of exposure are the same.

The quantitative aspects of assessing carcinogenic risks are discussed here because of the possible usefulness of this information in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the uncertainty of present estimations of cancer risks to humans at low levels of exposure should be recognized. The CAG feels that, given the limited data available from animal bioassays, especially at the high dosage levels required for testing, almost nothing can be known about the true shape of the dose-response curve at low environmental levels. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk is appreciably higher than the estimated risk, but it could very well be considerably lower. The risk estimates presented in this document should not, therefore, be regarded as accurate representations of the true cancer risks even when the exposures are accurately defined. These estimates may, however, be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

9.5.3.1.6. Alternative Methodological Approaches -- The methods used by the CAG for quantitative assessment are consistently conservative in that they tend to result in high estimates of risk. This conservatism is primarily due to the CAG's use of the linear nonthreshold extrapolation model in preference to any one of a variety of other extrapolation models that would give lower risk estimates. For purposes of comparison, descriptions of these alternative models (the one-hit, the probit, and the Weibull models) are presented in Appendix 9A.

Another method of risk estimation employed by the CAG involves the use of animal bioassay data as the basis for extrapolation. At present, the CAG's approach is to utilize data corresponding to the most sensitive animal responses in these studies. An alternative approach would be to use the average responses of all adequately tested bioassay animals.

Extrapolations from animals to humans can also be made on the basis of either relative weight or surface area. The latter approach, which is used by the CAG, has more of a basis in human pharmacological responses; however, at the present time there is some question as to which of the two approaches is more appropriate for use with carcinogens. Given this uncertainty, the CAG has chosen the most generally employed method, which is also the more conservative of the two. In the case of ethylene oxide inhalation studies, the use of extrapolation based on surface area rather than weight increases the unit risk estimates by a factor of 5.5 for the males and 6.8 for the females.

9.5.3.2. HUMANS--MODEL FOR ESTIMATION OF UNIT RISK BASED ON HUMAN DATA -- Whenever possible, the CAG utilizes data from human epidemiologic studies in preference to animal bioassay data. If sufficiently valid exposure information is available for a given compound, this information is always used by the CAG in its assessment. If the results of such studies show carcinogenic effects, the data are analyzed to give estimates of the linear dependence of cancer rates on lifetime average doses (equivalent to the factor B_H in the equation below). If human epidemiologic studies show no carcinogenic effects when positive animal evidence is available, then it is assumed that a risk does exist, but that the risk is smaller than could have been observed in an epidemiologic study. In such cases it is assumed that the true incidence is just below the level of detection in the cohort studied, and calculations are then made to estimate an upper limit of cancer incidence, as determined largely by the size of the cohort.

Very little information exists that can support extrapolation from high-exposure occupational studies to situations in which contamination is at low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, responses are measured in terms of the relative risk of an exposed cohort as compared to a control group. The mathematical model employed by the CAG assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), P_0, may be represented by the linear equation

$$P_0 = A + b_H X$$

where A is the lifetime probability of death from cancer in the absence of the agent, and X is the average lifetime exposure to environmental levels in units such as ppm. The factor, b_H, is the increased probability of cancer associated with each unit increase of the agent in air.

If it is assumed that R, the relative risk of lung cancer for exposed workers as compared to the general population, is independent of the length or age of exposure and depends only on average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + b_H\ (X_1+X_2)}{A + b_H\ (X_1)}$$

or

$$RP_0 = A + b_H\ (X_1 + X_2)$$

where X_1 = lifetime average daily exposure to the agent for the general population, X_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible ethylene oxide exposure. Substituting $P_0 = A + b_H X_1$ and rearranging gives

$$b_H = P_0\ (R - 1)/X_2$$

To use the above model, estimates of R and X_2 must be obtained from appropriate epidemiologic studies. The value of P_0 is derived by means of life-table methodology from 1976 U.S. vital statistics records of age- and cause-specific death rates for males. For leukemia, the estimate of P_0 is 0.0091. This methodology is utilized by the CAG in the present document, in the section on unit risk based on human data.

9.5.3.3. UNIT RISK ESTIMATES FOR ETHYLENE OXIDE

9.5.3.3.1. Unit Risk Estimate Based On Animal Studies -- The two long-term animal inhalation studies presented in the qualitative carcinogenicity section of this document showed similar results, both qualitatively and quantitatively, for the males. Both studies had significantly increased dose-related incidences of peritoneal mesotheliomas and gliomas, and some increase in mononuclear cell leukemias. These studies will be analyzed separately and then compared.

9.5.3.3.1.1. Snellings et al. (1981) (Bushy Run). This study exposed 120 Fischer 344 rats of each sex to three different doses (100 ppm, 33 ppm, and 10 ppm) of ethylene oxide vapor via inhalation for 6 hours/day, 5 days/ week, for approximately 2 years. Comparable untreated (air) control groups were also used. Interim sacrifices were conducted to evaluate the time development of treatment-related effects.

The results of the study show statistically significant increases in brain gliomas (highest dose group) and in mononuclear cell leukemias in females in the two highest dose groups, and peritoneal mesotheliomas and brain gliomas in males in the two highest dose groups. The above tumors all exhibited dose-response trends. Table 9-33 summarizes the pertinent data from this study which the CAG has used in calculating potency estimates for ethylene oxide. In connection with these data, it should be noted that the brain gliomas were not examined histopathologically until after the results of the NIOSH study (Lynch et al., 1982) had alerted the Bushy Run researchers to the possibility of the occurrence of brain neoplasias. For this reason, only 18-month, 24-month, and dead/euthanized moribund denominator figures were available for gliomas. For the male peritoneal mesotheliomas and the female mononuclear cell leukemias, the denominators in Table 9-33 correspond to the number of animals alive when the first tumor of that type was found. In the males, the first peritoneal mesothelioma was found at 15 months; in the females, the first mononuclear cell leukemia was found at 18 months (see

TABLE 9-33. BUSHY RUN ETHYLENE OXIDE INHALATION STUDY IN FISCHER 344 RATS. INCIDENCE OF PERITONEAL MESOTHELIOMA AND BRAIN GLIOMA IN MALES, AND MONONUCLEAR CELL LEUKEMIA AND BRAIN GLIOMA[a] IN FEMALES BY DOSE AMONG SURVIVORS TO FIRST TUMOR. MAXIMUM LIKELIHOOD ESTIMATES OF LINEAR TERM AND 95% UPPER-LIMIT q_1^*

	Exposure in air (ppm)				Linear term estimates	
Group	0 (combined)	10	33	100	MLE q_1	$q_1^{*\,b}$ $(mg/kg/day)^{-1}$
Males						
Peritoneal meso./No. examined (%)[c] p-values[d]	4/187(2) <0.00001	3/88(3)	7/82(8) =0.02	22/96(22) <0.0001	$5.1x10^{-2}$	$1.1x10^{-1}$
Brain gliomas/No. examined (%)[e] p-values	1/196(0.5) =0.0003	1/99(1)	5/98(5) =0.02	7/99(7) =0.002	$3.1x10^{-2}$	$5.0x10^{-2}$
Total p-values	5/187(3) <0.00001	4/88(5)	12/82(15) =0.0005	29/96(30) <0.0001	$1.1x10^{-1}$	$1.7x10^{-1}$
Human equivalent dose (mg/kg/day)[f]	0	0.35	0.94	2.63		
Females						
Mon. leukem/No. examined (%)[g] p-values	22/186(12) <0.00001	14/71(20) =0.08	24/72(33) 0.0001	28/73(38) <0.0001	$2.0x10^{-1}$	$2.9x10^{-1}$
Brain gliomas/No. examined (%)[e] p-values	1/194(0.5) =0.014	1/95(1)	3/99(3)	4/99(4) =0.05	$2.0x10^{-2}$	$4.0x10^{-2}$
Total p-values	23/186(12) <0.00001	15/71(21)	27/72(38) <0.0001	32/73(44) <0.0001	$2.5x10^{-1}$	$3.5x10^{-1}$
Human equivalent dose (mg/kg/day)[f]	0	0.28	0.75	2.11		

[a] See Table 9-27.
[b] 95% upper-limit unit risk estimate.
[c] Number alive at 15 months.
[d] Fisher Exact Test vs. combined controls (one tailed). P-value under controls is a one-sided Cochran-Armitage test for a dose-response trend.
[e] Total number examined less 6- and 12-month sacrifices.
[f] Based on measured doses in males of 20.24 and 2.7 mg/kg b.w. following 6 hours' exposure to ethylene oxide at 100 ppm and 10 ppm, respectively. The animal-to-human dose equivalences are based on a dose per surface area factor of $(70/W_a)^{1/3}$, which increases unit risk estimates by factors of 5.5 for the males and 6.8 for the females over dose per body weight equivalences.
[g] Number alive at 18 months.

SOURCE: Adapted from Snellings et al., 1981.

also Table 9-23).

As reported earlier, a dose of 20.24 mg/kg of body weight has been measured for male Fischer 344 rats exposed to ethylene oxide at 100 ppm under conditions similar to those of the Snellings et al. (1981) study. For this document, dose is assumed to be equivalent between species on the basis of mg/surface area, or mg/body weight$^{2/3}$. This means that a dose of 2.63 mg/kg body weight given to a 70 kg human is assumed to produce an equivalent response to that produced by 20.24 mg/kg in the male rat. As discussed above and as shown in Table 9-33, this method of determining dose equivalence increases the unit risk estimates by factors of 5.5 for females and 6.8 for males over estimates obtained on the basis of mg/kg of body weight.

Table 9-33, in presenting the total number of significant tumors by sex, sums the total number of significant tumors over the smallest denominator. This is done because time-to-tumor data on the gliomas are unavailable. Compared with the usual CAG procedure of counting the total number of animals with significant tumors, the addition of total significant tumors, as is done here, increases the risk estimate very slightly.

Calculations of the 95% upper-limit unit risk estimate, based on the linearized multistage model fitted to the data in Table 9-33, yield a high value of $q_h^* = 3.5 \times 10^{-1}(\mathrm{mg/kg/day})^{-1}$, based on total mononuclear cell leukemias and brain gliomas in the female rats. The responses of the males, based on total peritoneal mesotheliomas and brain gliomas, yield a value of 50% less, $q_h^* = 1.7 \times 10^{-1}(\mathrm{mg/kg/day})^{-1}$. The higher estimate is chosen for safety purposes.

To convert the above estimate to units of $\mu g/m^3$ for humans, the following formula is used:

$$1 \text{ mg/kg/day} = 1 \text{ mg/kg/day} \times 70 \text{ kg} \times 1000 \mu\text{g/mg} \times \text{day}/20 \text{ m}^3 = 3.5 \times 10^3 \mu\text{g/m}^3$$

or

$$1 \mu\text{g/m}^3 = 2.86 \times 10^{-4} \text{ mg/kg/day}.$$

The 95% upper-limit slope estimate in terms of μg/m^3 is thus calculated as

$$q_h^* = 3.5 \times 10^{-1} (\text{mg/kg/day})^{-1} \times 2.86 \times 10^{-4} \frac{(\text{mg/kg/day})}{\mu\text{g/m}^3} = 1.0 \times 10^{-4} (\mu\text{g/m}^3)^{-1}$$

To convert from μg/m^3 to ppm, the formula is

$$1 \text{ ppm} = \frac{1.2 \text{ g}}{10^{-3} \text{ m}^3} \times \frac{44.1 \text{ m.w. ethylene oxide}}{28.2 \text{ m.w. air}} \times \frac{10^6 \mu\text{g}}{\text{g}} \times 10^{-6}$$

$$= 1.9 \times 10^3 \mu\text{g/m}^3$$

The lifetime probability of cancer from continuously breathing 1 ppm ethylene oxide in air is thus calculated as follows:

$$P = 1.0 \times 10^{-4} (\mu\text{g/m}^3)^{-1} \times \frac{1.9 \times 10^3 \mu\text{g/m}^3}{\text{ppm}} = 1.9 \times 10^{-1} (\text{ppm})^{-1}$$

9.5.3.3.1.2. Lynch et al. (1982) (NIOSH). The NIOSH study (Lynch et al., 1982) in which male Fischer 344 rats were exposed to ethylene oxide at 50 ppm and 100 ppm 7 hours/day, 5 days/week for 2 years, produced results very similar to those of the Bushy Run study (Snellings et al., 1981). The results, shown in Table 9-34, show statistically significant increases and dose-response trends in brain gliomas and peritoneal mesotheliomas; there is a significant increase in mononuclear cell leukemias only at the lower dose, and no significant dose-response trend. Since incidence of this leukemia in controls was over 30% in this study, and since the Snellings et al. (1981)

study did not show a significant increase in these leukemias, only peritoneal mesotheliomas and brain gliomas were used for risk assessment. The results of the potency calculations, shown in Table 9-34, are quantitatively nearly identical to those in Table 9-33. Based on the above analyses, the maximum animal 95% upper-limit slope potency value is still $q_1^* = 3.5 \times 10^{-1}$(mg/kg/day)$^{-1}$ based on the total mononuclear cell leukemias and brain gliomas in female rats in the Snellings et al. study.

9.5.3.3.1.3. Effects of Results on Different Dose Equivalence Assumptions --OSHA Versus EPA Assessments. The results of the above assessments depend, to some extent, on the dose equivalence assumptions. Dose equivalence in the following discussion means the dose that will cause an equivalent response, quantitatively, in both species. The CAG has assumed that doses are equivalent on the basis of mg per surface area, an assumption for which there is some experimental evidence when first-order kinetics apply; for ethylene oxide, first-order kinetics appear to apply at exposures up to 100 ppm (Tyler and McKelvey, 1980). As explained in an earlier section, use of the surface area correction increases the 95% upper-limit unit risk estimate by factors of 5.5 for the males and 6.8 for the females over estimates obtained on the basis of mg/kg/body weight.* OSHA, which assumes equivalence on a mg/kg/body weight basis, calculated exposures of 19.30 mg/kg/day for males and 23.94 mg/kg/day for females exposed to ethylene oxide at 100 ppm in the Snellings et al. (1981) study, using EPA methodology (Federal Register 48[78]:

---

*Equivalence could also have been calculated directly on a ppm basis; this would have yielded a 95% upper-limit estimate approximately 1.8 times as high as that obtained on the basis of mg/kg/body weight. EPA uses direct ppm equivalence for partially soluble gases and particulates. Ethylene oxide can be considered a completely soluble gas.

TABLE 9-34. NIOSH ETHYLENE OXIDE INHALATION STUDY IN MALE FISCHER 344 RATS. INCIDENCE OF PERITONEAL MESOTHELIOMA AND BRAIN GLIOMA[a] BY DOSE, AMONG TOTAL EXAMINED. ESTIMATES OF 95% UPPER-LIMIT RISK BASED ON HUMAN EQUIVALENT DOSE (mg/kg/day)

	Exposure in air (ppm)			
	0[e]	50	100	q_1^* $(\text{mg/kg/day})^{-1}$
Peritoneal mesothelioma/No. examined	3/78[b]	9/79	21/79[b]	1.0×10^{-1}
Brain glioma/No. examined	0/76[c]	2/77	5/79[d]	3.4×10^{-2}
Total	3/78[b]	11/79[b]	26/79[b]	1.3×10^{-1}
Human equivalent dose (mg/kg/day)[f]	0	1.59	3.06	--

[a] See Table 9-29.
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$.
[e] p-values noted beside control incidences represent values associated with a one-sided Cochran-Armitage test for a dose-response trend.
[f] Human equivalent dose based on transforming ppm to mg/kg/day as in Table 9-33, except for an adjustment for 7 hours' exposure.

SOURCE: Lynch et al., 1982.

172-193) for a completely soluble gas. While the results for the males, 19.30 mg/kg/day, are within 5% of the dose measured by Tyler and McKelvey (1980) (20.24 mg/kg/day), EPA used the more accurate measured dose in this case. EPA then used the surface area correction factors for animal-to-man equivalence. Thus, on the basis of the difference in assumptions of equivalent dose alone, the EPA risk numbers are larger than OSHA's by a factor of about 6.

One other difference between the OSHA and EPA assessments, both based on the Bushy Run data, is that the EPA added total significant tumors (mononuclear cell leukemias and brain gliomas for the females), while OSHA used the total number of malignant tumor-bearing animals. For EPA, this led to factors higher by 50% for the males and 20% for the females. The result, based on animal data, is that the EPA 95% upper-limit unit risk factor is larger than that of OSHA by a factor of about 8.

9.5.3.3.2. Comparison of Animal and Human Inhalation Studies -- The purpose of this section is to determine whether or not the extrapolated risks from the animal data can reasonably predict the observed human results. As presented in Sections 9.5.1 and 9.5.2, there is strong evidence for the carcinogenicity of ethylene oxide in rats, while the evidence in humans is limited, but suggestive of a leukemia effect. Table 9-35 summarizes the leukemia evidence in humans. As can be seen, two of the four mortality studies reviewed had statistically significant increases in the relative risks of leukemia. None of the four studies was particularly revealing, however, since both the observed and expected numbers were quite small. Because the expected numbers were so small, the 95% confidence limits around the relative risk are quite large. Even with statistically significant increased cancers, estimates of

TABLE 9-35. LEUKEMIA (ICD 204-207) INCIDENCE AND MORTALITY: ETHYLENE OXIDE EPIDEMIOLOGY. INCLUDED ARE RELATIVE RISKS, 95% CONFIDENCE LIMITS, NOMINAL EXPOSURE ESTIMATES, AND 95% CONFIDENCE LIMITS ON UNIT RISK

Study	N	Obs.	Exp.	Relative risk	(95% confidence limits)	Exposure (ppm)	95% confidence limits on unit risk[a] (ppm^{-1})
Hogstedt (sterilant plant) (1968-1977)							
Storage hall	(70)	2	0.03	66.7	8-240	20 ± 10 (8 hr TWA)	0.07 - 2.5
Adjacent area	(160)	0	0.07	0	0-52	"exposed occasionally on passing through"	No other estimates possible
Total (original, 1968-77)	(230)	2	0.10	20	2.4-72		
(New data, 1968-1982) ICD 200-207	(203)[b]	4	0.3	13.3	3.6-34.2		
Hogstedt (prod. plant) a. (1961-1982) ≥ 10 yr latency ≥ 1 yr exposure							
Direct exposure	(89)	2	0.18	10.0	1.3-40.1	< 14	
Intermittent exposure	(79)	1	0.16	6.2	0.1-42.8	"less exposure"	
b. ≥ 10 yr exposure ≥ 20 yr induction-latency (1961-1977)							
Direct exposure		1	0.04	25	0.3-139		
Intermittent exposure		1	0.1	10	0.1-55.6		
Morgan et al. (1955-1977)	(767)	0	0.7	0	0-5.2	< 10	
Thiess et al. (1928-1980) ≥ 10 yr exposure	(351)	1	0.15	6.8	0.1-37	mostly < 5 ppm (a few large variations)	

---

[a] See text. Estimates for most groups not possible, due to lack of information about exposure duration.

[b] Includes those employed at least one year. Hogstedt et al. (1984) subcohorts constructed differently than earlier Hogstedt et al. (1979b) subcohorts.

hazard due to ethylene oxide are difficult because of the small sample size.

An even greater problem associated with determining potency estimation from human studies is the general lack of exposure information. Table 9-35 presents some ethylene oxide measurements based on either 8-hour time-weighted averages or spot measurements, but other important data are generally missing. Such necessary information as average length of exposure, average age at exposure, and average length of follow-up cannot even be estimated from three of the four studies in the literature. Only the subcohort of storage hall workers in the sterilant plant (Hogstedt et al., 1979b, 1984) is consistent enough in terms of exposure conditions, duration, and follow-up to estimate 95% confidence limits of ethylene oxide potency. This is presented in the following paragraphs. The simplifying assumptions add to the uncertainty of the estimate.

The risk assessment done on the basis of the Hogstedt et al. (1979b) study probably underestimates the carcinogenic potency of ethylene oxide because of two factors: 1) In this study, exposure started in 1968 and ended in 1977--giving a maximum latency period of only 9 years, whereas cancer usually involves a relatively long latency period. (However, the author states that leukemia incidence in Hiroshima and Nagasaki due to the atomic bomb irradiation showed a rapid increase that began shortly after exposure and reached a peak after 6 years.) 2) Since the study did not report the number of person-years of exposure, it is assumed for present purposes that all of the 230 workers were exposed for the full 9 years, an assumption which tends to underestimate the risk. Another problem with this study is that the gas used for sterilization was 50% ethylene oxide and 50% methyl formate. Little is known about the biological effects of methyl formate or of the combination of methyl formate with ethylene oxide. However, methyl formate is known to metabolize to formic acid, which is a normal body metabolite. It is assumed for present purposes that ethylene oxide was the only leukemogen in this study, although one of the cases (the man) had reported some contact with benzene in laboratory work.

Hogstedt et al. (1979b) states, in connection with exposures in the factory studied, that infrared spectrophotometry and gas chromatography measurements in 1977 showed values ranging from 2 to 70 ppm in the factory's storage hall area. The study also reports that the calculated 8-hour time-weighted average ethylene oxide concentration in the breathing zone was 20 ± 10 ppm, and that the concentration in the storage hall was higher than in the sterilization room. The accompanying table described the 70 storage hall employees as having had 8-hour exposures, while all but seven of the remaining employees were described as "occasionally exposed."

Of the two leukemia cases (acute myeloid and chronic myeloid), both people worked in the storage hall area, and neither had reported exposure to benzene. Because the two cases worked in the storage hall, the CAG has chosen to estimate the expected number of leukemia cases for the persons who worked only in that area rather than in the entire factory. Based on the reported expected leukemia incidence of 0.1 cases for the 230 exposed employees, we can estimate approximately (70/230) x 0.1 = 0.03 cases for the group exposed in the storage hall. Compared with the two observed cases, this yields a ratio of observed to expected cases of (2/0.03) = 66.7.

The estimated average exposure to ethylene oxide over the lifetime of the workers is calculated as follows:

$$20 \text{ ppm} \times 8/24 \text{ hr} \times 240/365 \text{ days} \times 9/45.6 \text{ yr}$$

$$\text{exposure} = 0.865 \text{ ppm}$$

where 45.6 years is the mean age of the 70 storage hall employees at the end of the study period.

The slope b_H of the lifetime probability of dying from leukemia due to a lifetime of breathing ethylene oxide at 1 ppm (Section 9.5.3.2.) is given by

$$b_H = \frac{P_0 \, (R - 1) \, X_1}{X_2}$$

where P_0 is the lifetime probability of dying* from leukemia in the United States in the absence of ethylene oxide exposure, R is the relative risk, X_1 is the exposure of 1 ppm, and X_2 is the exposure experienced by the factory workers. The relative risk R estimated above is 66.7; the exposure X_2 is given as 0.865 ppm. The lifetime probability of death from leukemia in the U.S. population is 0.0091. Substituting these values in the above equation gives

$$b_H = \frac{0.0091 \, (66.7 - 1)}{0.865 \text{ ppm}} = 0.69(\text{ppm})^{-1}$$

The probability associated with breathing ethylene oxide at 1 ppm for a lifetime is

$$P = 1 - e^{-b_H (1 \text{ ppm})} = 0.50$$

To convert ppm to $\mu g/m^3$, the formula is

---

*P_0 employs both leukemia incidence cases and leukemia mortality rates. While leukemia mortality in the younger ages (< 55) can be closely equated with incidence, in the older age groups chronic forms predominate in incidence, with death often occurring from other causes. Nevertheless, for this assessment it is assumed that although ethylene oxide would cause all types of leukemias, death will result from each case. In this study, the leukemias in the two women were of the acute form.

$$1\ \text{ppm} = \frac{1.2\ \text{g}}{10^{-3}\ \text{m}^3} \times \frac{44.1\ \text{m.w. chemical}}{28.2\ \text{m.w. air}} \times \frac{10^6\ \mu\text{g}}{\text{g}} \times 10^{-6}$$

$$= 1.9 \times 10^3\ \mu\text{g/m}^3$$

Thus the unit risk estimate in terms of μ g/m^3 is

$$b_H = 0.69(\text{ppm})^{-1} \times \frac{1\ \text{ppm}}{1.9 \times 10^3\ \mu\text{g/m}^3} = 3.6 \times 10^{-4}(\mu\text{g/m}^3)^{-1}$$

Similarly, if the 95% limits on the relative risk from Table 9-35 are substituted for the point estimate, the 95% confidence limits on the unit risk become $7.0 \times 10^{-2}(\text{ppm})^{-1}$ to $2.5(\text{ppm})^{-1}$. This represents a range of 36 and encompasses the 95% upper-limit incremental unit risk, $q_1^* = 1.9 \times 10^{-1}$ ppm^{-1}, extrapolated from the Snellings et al. (1981) study.

Based on the above analysis, we conclude that the carcinogenic potency estimates for ethylene oxide derived from human data do not contradict the estimate based on the rat inhalation studies. Because of the uncertainties in the epidemiologic study, however, the animal inhalation study is chosen for the 95% upper-limit incremental unit risk estimate for ethylene oxide.

9.5.3.4. RELATIVE POTENCY -- One of the uses of the concept of unit risk is to compare the relative potencies of carcinogens. For the purposes of the present analysis, potency is defined as the linear portion of the dose-response curve, and is used to calculate the required unit risk factors. To estimate relative potency on a per-mole basis, the unit risk slope factor is multiplied by the molecular weight of the compound, and the resulting number, expressed in terms of (mmol/kg/day)$^{-1}$, is called the "relative potency index."

Figure 9-4 is a histogram representing the frequency distribution of relative potency indices for 54 chemicals that have been evaluated by the CAG as

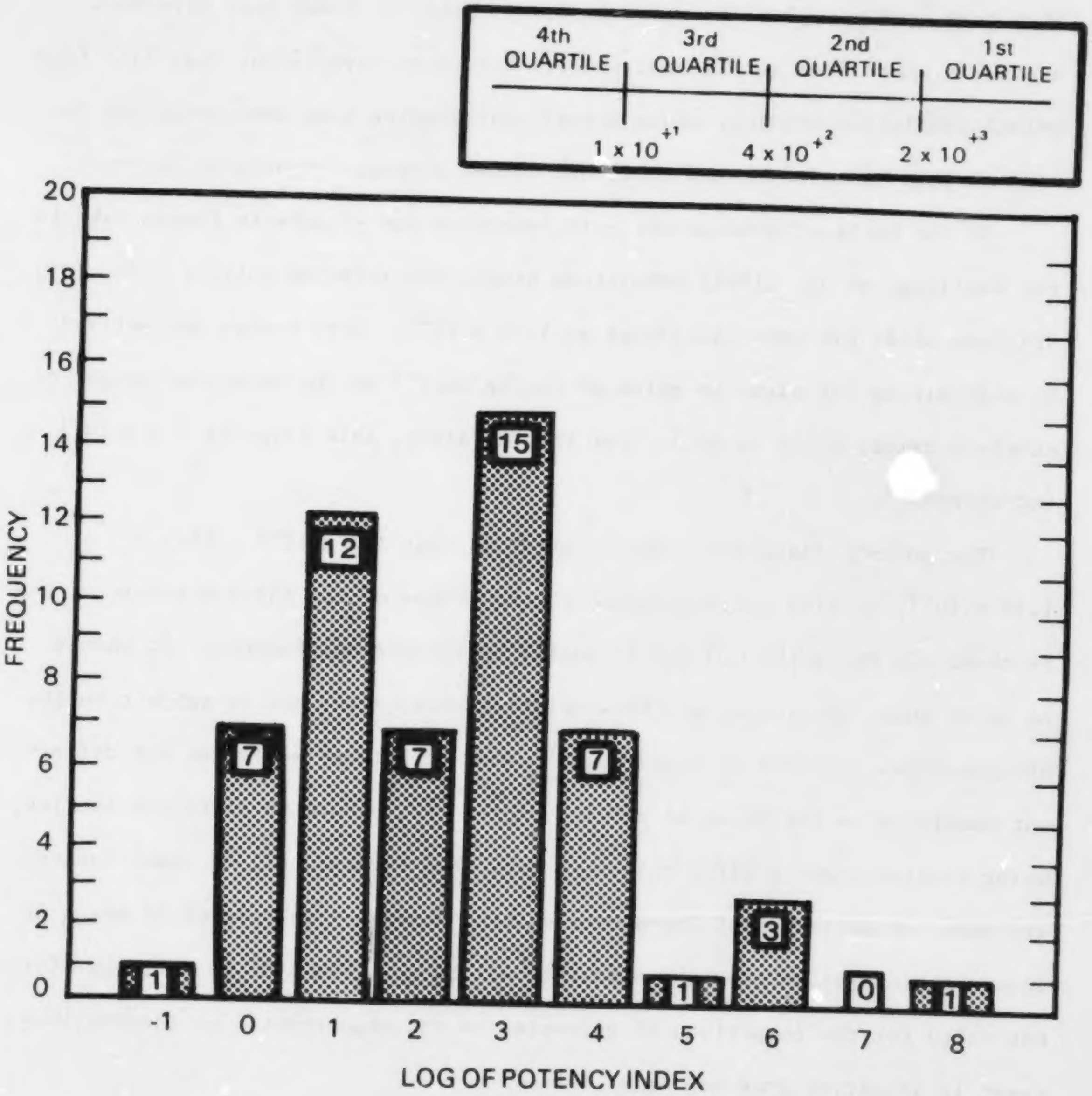


Figure 9-4. Histogram representing the frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

suspect carcinogens. The data summarized by the histogram are presented in Table 9-36. Where human data have been available for a compound, such data have been used to calculate these indices. Where no human data have been available, data from animal oral studies have been used rather than data from animal inhalation studies, since animal oral studies have been conducted for most of these compounds, and their use allows potency comparisons by route.

On the basis of mononuclear cell leukemias and gliomas in female rats in the Snellings et al. (1981) inhalation study, the relative potency index for ethylene oxide has been calculated as $1.54 \times 10^{+1}$. This number was derived by multiplying the slope in units of $(mg/kg/day)^{-1}$ by the molecular weight of ethylene oxide, which is 44.1. For the rat study, this slope is 3.5×10^{-1} $(mg/kg/day)^{+1}$.

The potency index for ethylene oxide is thus $3.5 \times 10^{-1} \times 44.1 = 1.54 \times 10^{-1}$, putting ethylene oxide at the bottom of the third quartile of the 54 chemicals which the CAG has evaluated as suspect carcinogens. It should be noted that the ranking of these relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals on the basis of varying routes of exposure in different species, using studies whose quality varies widely. Furthermore, all of these indices are based on estimates of low-dose risk that have been calculated by means of linear extrapolation from the observational range. The indices are, therefore, not valid for the comparison of potencies in the experimental or observational range if linearity does not exist there.

9.5.4. Summary. Ethylene oxide has been shown to be carcinogenic in animals in long-term studies by three different routes of administration (inhalation, subcutaneous injection, and gavage). The most relevant route for human expo-

TABLE 9-36. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	1162-65-8	L	S	2A	2900	312.3	$9x10^{+5}$	+6
Aldrin	309-00-2	I	L	2B	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride	107-05-1				$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	1	$2.9x10^{-2}$(W)	78	$2x10^{0}$	0
Benzidene	92-87-5	S	S	1	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	7440-41-7	L	S	2A	2.6	9	$2x10^{+1}$	+1
1,3-Butadiene	106-99-0	I	S	2B	$1.0x10^{-1}$(I)	54.1	$5x10^{0}$	+1
Cadmium	7440-43-9	L	S	2A	7.8(W)	112.4	$9x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	2B	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	$7x10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 9-36. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
Chlorinated ethanes								
1,2-Dichloroethane	107-06-2	I	S	2B	6.9×10^{-2}	98.9	7×10^{0}	+1
hexachloroethane	67-72-1	I	L	3	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-Tetrachloroethane	79-34-5	I	L	3	0.20	167.9	$3 \times 10^{+1}$	+1
1,1,2-Trichloroethane	79-00-5	I	L	3	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	67-66-3	I	S	2B	7×10^{-2}	119.4	8×10^{0}	+1
Chromium VI	7440-47-3	S	S	1	41(W)	100	$4 \times 10^{+3}$	+4
DDT	50-29-3	I	S	2B	0.34	354.5	$1 \times 10^{+2}$	+2
Dichlorobenzidine	91-94-1	I	S	2B	1.69	253.1	$4 \times 10^{+2}$	+3
1,1-Dichloroethylene (Vinylidene chloride)	75-35-4	I	L	3	1.17(I)	97	$1 \times 10^{+2}$	+2
Dichloromethane (Methylene chloride)	75-09-2	I	L	3	6.3×10^{-4}(I)	84.9	5×10^{-2}	-1
Dieldrin	60-57-1	I	S	2B	30.4	380.9	$1 \times 10^{+4}$	+4
2,4-Dinitrotoluene	121-14-2	I	S	2B	0.31	182	$6 \times 10^{+1}$	+2
Diphenylhydrazine	122-66-7	I	S	2B	0.77	180	$1 \times 10^{+2}$	+2
Epichlorohydrin	106-89-8	I	S	2B	9.9×10^{-3}	92.5	9×10^{-1}	0
Bis(2-chloroethyl)ether	111-44-4	I	S	2B	1.14	143	$2 \times 10^{+2}$	+2

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 9-36. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Bis(chloromethyl)ether	542-88-1	S	S	1	9300(I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	$8x10^{+3}$	+4
Ethylene oxide	75-21-8	L	S	2A	$3.5x10^{-1}$(I)	44.1	$2x10^{+1}$	+1
Heptachlor	76-44-8	I	S	2B	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	3	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					4.75	290.9	$1x10^{+3}$	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	$3x10^{+3}$	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	58-89-9	I	L	2B	1.33	290.9	$4x10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	$6.2x10^{+3}$	391	$2x10^{+6}$	+6
Nickel	7440-02-0	L	S	2A	1.15(W)	58.7	$7x10^{+1}$	+2
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	$9x10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 9-36. (continued)

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	$4x10^{+3}$	+4
N-nitroso-N-methylurea	684-93-5	I	S	2B	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	2B	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	1336-36-3	I	S	2B	4.34	324	$1x10^{+3}$	+3
Phenols								
2,4,6-Trichlorophenol	88-06-2	I	S	2B	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	2B	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
Tetrachloroethylene	127-18-4	I	L	3	$6.0x10^{-2}$	165.8	$1x10^{1}$	+1
Toxaphene	8001-35-2	I	S	2B	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	79-01-6	I	L/S	3/2B	$1.2x10^{-2}$	131.4	$2x10^{0}$	0
Vinyl chloride	75-01-4	S	S	1	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model.

2. The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

sure is inhalation. Two long-term inhalation studies in rats were performed that adequately tested the carcinogenic potential of ethylene oxide by inhalation: the Bushy Run study (Snellings et al., 1981) and the NIOSH study (Lynch et al., 1982). Snellings et al. (1981) found that ethylene oxide exposure resulted in an increased incidence of mononuclear cell leukemia in females in the two highest dose groups; this increase was dose related. The test for linear trend was highly significant ($p < 0.0001$). There was also a significant ($p = 0.045$) increase in gliomas at the highest dose, and the test for linear trend was highly significant ($p = 0.014$). In males, incidences of primary brain neoplasm, peritoneal mesothelioma, and subcutaneous fibroma were significantly elevated in at least two exposed groups. The trend analysis was significant for both mesotheliomas ($p < 0.00001$) and gliomas ($p = 0.003$) in males. In the NIOSH (Lynch et al., 1982) study, which involved only male rats, leukemia incidence was significantly increased at low doses only, while gliomas (mixed-cell) and peritoneal mesotheliomas were increased significantly in the high-dose groups. For these latter two sites, the dose-response trend tests were also statistically significant ($p < 0.01$). Other positive results for the carcinogenicity of ethylene oxide were demonstrated by subcutaneous injection in mice and intragastric administration in rats.

Three epidemiologic studies of workers exposed to ethylene oxide demonstrated significant ($p < 0.05$) association between ethylene oxide exposure and the occurrence of cancer. Two of the studies (Hogstedt et al., 1979a, b) found an association between ethylene oxide exposure and the incidence of leukemia. Ethylene oxide was not found to be associated with any particular type of leukemia, however. Other sites or types of cancer found to be significantly ($p < 0.05$) associated with ethylene oxide exposure in an individual study include pancreatic cancer and Hodgkin's disease in the Morgan et al.

(1981) study and stomach cancer in the Hogstedt et al. (1979a) study. The possibility of confounding due to other chemical agents cannot be excluded in any of the studies, however.

An upper-limit incremental unit risk estimate of $1.0 \times 10^{-4} (\mu g/m^3)^{-1}$ for ethylene oxide has been calculated, using a linearized multistage model, on total mononuclear cell leukemias and brain gliomas in female Fischer 344 rats from the Bushy Run (Snellings et al., 1981) study. Extrapolation from the human leukemia data results in a highly uncertain risk estimate due to the small numbers of leukemia cases that were observed and expected. Quantitative comparisons of human and animal inhalation studies do, to the extent possible, support each other.

9.5.5. Conclusions. Ethylene oxide has been shown to be carcinogenic in animals by intragastric, subcutaneous injection, and inhalation routes of exposure. Three human studies show an association between ethylene oxide exposure and an excess risk of cancer, but each of these studies has some limitations. Other evidence, which is in the mutagenicity section of this document, supports the conclusions for carcinogenicity in that ethylene oxide is a direct-acting alkylating agent, it reacts with mammalian DNA, it induces base-pair substitutions in the Ames test and gene mutations in plants and animals, and it breaks chromosomes of plants, animals, and humans and causes DNA damage in the spermatids of mice.

Using the weight-of-evidence criteria of EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984), the Carcinogen Assessment Group considers the animal evidence for carcinogenicity to be "sufficient" and the human evidence to be "limited" bordering on inadequate. Based on both the animal and human findings, the overall EPA classification for ethylene oxide is Group B1, meaning that ethylene oxide should be considered as probably carcinogenic to humans. This B1 classification is qualified as bordering on Group B2 because of the limitations in the human evidence.

According to the IARC guidelines for evaluating carcinogen evidence (See Appendix 9B), ethylene oxide would be classified in Group 2A. This classification is similarly qualified as bordering on Group 2B because of limitations in the human evidence. A Group 2 classification, whether 2A or 2B, means that ethylene oxide should be considered as probably carcinogenic in humans.

An upper-limit carcinogenic potency value of 3.5×10^{-1} $(mg/kg/day)^{-1}$ has been calculated based on total mononuclear cell leukemias and brain gliomas in female Fischer 344 rats in the Snellings et al. (1981) study. An upper-limit incremental unit risk of 1.0×10^{-4} $(\mu g/m^3)^{-1}$ has also been estimated using a linearized multistage model.

APPENDIX 9A

COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimate of unit risk from animals presented in the body of this document was calculated by use of the linearized multistage model. This nonthreshold model is part of a methodology for estimating a conservative linear slope at low extrapolation doses that is usually consistent with the data at all dose levels in an experiment. The model holds that the most plausible upper limits of risk are those predicted by linear extrapolations to low levels of the dose-response relationship.

Other nonthreshold models that have been used for risk extrapolation are the one-hit, the log-Probit, and the Weibull models. The one-hit model is characterized by a continuous downward curvature, but is linear at low doses. Because of its functional form, the one-hit model can be considered the linear form or first stage of the multistage model. This fact, together with the downward curvature of the one-hit model, means that the model will always yield low-level risk estimates that are at least as large as those obtained with the multistage model. In addition, whenever the data can be fitted adequately to the one-hit model, estimates based on the one-hit model and the multistage model will be comparable.

The log-Probit and the Weibull models, because of their general "S" curvature, are often used for the interpretation of toxicological data in the observable range. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-Probit model was originally used in biological assay problems such as potency assessments of toxicants and drugs, and is most often used to estimate such values as percentile lethal dose or percentile effective dose. The log-Probit model was developed along strictly empirical lines, in studies where it was observed that several log dose-response relationships followed the cumulative normal probability distribution function, Φ. In fitting the log-Probit model to cancer bioassay data, assuming an independent background, this relationship becomes

$$P(D;a,b,c) = c + (1-c)\ \Phi\ (a+b\log_{10} D) \qquad a,b > 0 < c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individual tolerances, and b is an estimate of the log-Probit dose-response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a quantifiable probability of transforming a single normal cell into a cancer cell. In this model, the probability distribution function is

$$P(D;a,b) = 1-\exp-(a+bd) \qquad a,b > 0$$

where a and b are the parameter estimates (a = the background or zero dose rate, and b = the linear component or slope of the dose-response model). In considering the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \qquad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model, known as the Weibull model, is of the form

$$P(D;b,k) = 1-\exp-(bd^{k}) \qquad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, the model yields low-dose estimates of risks that are usually significantly lower than either the multistage or one-hit models, both of which are linear at low doses. All three of these models--the multistage, the one-hit, and the Weibull--usually project risk estimates that are significantly higher at low exposure levels than those projected by the log-Probit model.

The results of both the male and female rat data sets from the Bushy Run (Snellings et al., 1981) study are presented in Table 9A-1. Surprisingly, for the female rats, both the Weibull and log-Probit models yielded larger estimates of risk than the multistage model, which, in this case, produced results identical to those produced by the one-hit model. For the males, the one-hit model produced the highest estimates and the log-Probit model produced the lowest; in this case, the multistage, one-hit, and Weibull all produced similar results.

TABLE A-1. ESTIMATES OF HUMAN LOW-DOSE RISK BASED ON DATA FROM MALE AND FEMALE FISCHER 344 RATS IN THE BUSHY RUN ETO INHALATION STUDY, AS DERIVED FROM FOUR DIFFERENT MODELS. ALL ESTIMATES INCORPORATE ABBOTT'S CORRECTION FOR INDEPENDENT BACKGROUND RATE

Continuous human exposure ppm	Maximum likelihood estimates of additional risks				95% upper confidence limit of additional risks			
	Multistage model	One-hit model	Weibull model	Log-Probit model	Multistage model	One-hit model	Weibull model	Log-Probit model
Males								
.001	$3.1x10^{-4}$	$1.3x10^{-4}$	$2.5x10^{-5}$	$3.1x10^{-1}$	$9.2x10^{-5}$	$1.6x10^{-4}$	$1.3x10^{-4}$	$5.0x10^{-9}$
0.01	$3.1x10^{-3}$	$1.3x10^{-3}$	$3.3x10^{-4}$	$1.6x10^{-6}$	$9.2x10^{-4}$	$1.6x10^{-3}$	$1.4x10^{-3}$	$1.5x10^{-5}$
0.1	$3.1x10^{-2}$	$1.3x10^{-2}$	$4.3x10^{-3}$	$8.6x10^{-4}$	$9.2x10^{-3}$	$1.6x10^{-2}$	$1.3x10^{-2}$	$3.9x10^{-3}$
1	$8.4x10^{-2}$	$1.2x10^{-1}$	$5.5x10^{-2}$	$5.4x10^{-2}$	$8.8x10^{-2}$	$1.6x10^{-1}$	$9.7x10^{-2}$	$9.7x10^{-2}$
Females								
.001	$1.4x10^{-5}$		$3.6x10^{-3}$	$1.2x10^{-4}$	$1.9x10^{-4}$		$1.5x10^{-2}$	$1.0x10^{-3}$
0.01	$1.4x10^{-3}$		$1.4x10^{-2}$	$3.0x10^{-3}$	$1.9x10^{-3}$		$4.3x10^{-2}$	$1.5x10^{-2}$
0.1	$1.4x10^{-2}$		$5.0x10^{-2}$	$3.3x10^{-2}$	$1.9x10^{-2}$		$1.1x10^{-1}$	$9.2x10^{-2}$
1	$1.3x10^{-1}$		$1.7x10^{-1}$	$1.7x10^{-1}$	$1.7x10^{-1}$		$2.5x10^{-1}$	$2.5x10^{-1}$

Animal exposure 0, 33 ppm, 100 ppm 6 hours/day, 5 days/week.

DATA

	Human eq. dose - mg/kg/day					Human eq. dose - mg/kg/day			
Males	0	0.35	0.94	2.63	**Females**	0	0.28	0.75	2.11
No. tumors/No. examined	5/187	4/88	12/82	29/96		23/186	15/71	27/72	32/73

Conversions for low doses: Humans 1 mg/kg/day = 1.84 ppm in air

or 1 ppm air = .543 mg/kg/day

Multistage and one-hit models gave identical results in females.

APPENDIX 9B

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER CLASSIFICATION SYSTEM FOR THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS*

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN HUMANS

Evidence of carcinogenicity from human studies comes from three main sources:

1. Case reports of individual cancer patients who were exposed to the chemical or process.
2. Descriptive epidemiological studies in which the incidence of cancer in human populations was found to vary in space or time with exposure to the agents.
3. Analytical epidemiological (case-control and cohort) studies in which individual exposure to the chemical or group of chemicals was found to be associated with an increased risk of cancer.

Three criteria must be met before a causal association can be inferred between exposure and cancer in humans:

1. There is no identified bias which could explain the association.
2. The possibility of confounding has been considered and ruled out as explaining the association.
3. The association is unlikely to be due to chance.

In general, although a single study may be indicative of a cause-effect relationship, confidence in inferring a causal association is increased when several independent studies are concordant in showing the association, when the association is strong, when there is a dose-response relationship, or when a reduction in exposure is followed by a reduction in the incidence of cancer.

---

*Adapted from International Agency for Research on Cancer. Monographs Supplement 4, Evaluation of the Carcinogenic Risk of Chemicals to Humans, 1982, pp. 11-14.

The degrees of evidence for carcinogenicity from studies in humans were categorized as:

1. Sufficient evidence of carcinogenicity, which indicates that there is a causal relationship between the agent and human cancer.

2. Limited evidence of carcinogenicity, which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded.

3. Inadequate evidence, which indicates that one of three conditions prevailed: (a) there were few pertinent data; (b) the available studies, while showing evidence of association, did not exclude chance, bias, or confounding; (c) studies were available which do not show evidence of carcinogenicity.

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN EXPERIMENTAL ANIMALS

These assessments were classified into four groups:

1. Sufficient evidence of carcinogenicity, which indicates that there is an increased incidence of malignant tumors: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.

2. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) the studies involve a single species, strain, or experiment; (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting; or (c) the neoplasms produced often occur spontaneously and, in the past, have been difficult to classify as malignant by histological criteria alone (e.g., lung and liver tumors in mice).

3. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect; or that within the limits of the tests used, the chemical is not carcinogenic. The number of negative studies is small, since, in general, studies that show no effect are less likely to be published than those suggesting carcinogenicity.

4. No data indicates that data were not available to the Working Group.

The categories sufficient evidence and limited evidence refer only to the strength of the experimental evidence that these chemicals are carcinogenic and not to the extent of their carcinogenic activity nor to the mechanism involved. The classification of any chemical may change as new information becomes available.

EVALUATION OF CARCINOGENIC RISK TO HUMANS

At present, no objective criteria exist to interpret data from studies in experimental animals or from short-term tests directly in terms of human risk. Thus, in the absence of sufficient evidence from human studies, evaluation of the carcinogenic risk to humans was based on consideration of both the epidemiological and experimental evidence. The breadth of the categories of evidence defined above allows substantial variation within each. The decisions reached by the Working Group regarding overall risk incorporated these differences, even though they could not always be reflected adequately in the placement of an exposure into a particular category.

The chemicals, groups of chemicals, industrial processes, or occupational exposures were thus put into one of three groups:

Group 1

The chemical, group of chemicals, industrial process, or occupational exposure is carcinogenic to humans. This category was used only when there was sufficient evidence from epidemiological studies to support a causal association between the exposure and cancer.

Group 2

The chemical, group of chemicals, industrial process, or occupational exposure is probably carcinogenic to humans. This category includes exposures for which, at one extreme, the evidence of human carcinogenicity is almost "sufficient," as well as exposures for which, at the other extreme, it is inadequate. To reflect this range, the category was divided into higher (Group A) and lower (Group B) degrees of evidence. Usually, category 2A was reserved for exposures for which there was at least limited evidence of carcinogenicity to humans. The data from studies in experimental animals played an important role in assigning studies to category 2, and particularly those in Group B; thus, the combination of sufficient evidence in animals and inadequate data in humans usually resulted in a classification of 2B.

In some cases, the Working Group considered that the known chemical properties of a compound and the results from short-term tests allowed its transfer from Group 3 to 2B or from Group 2B to 2A.

Group 3

The chemical, group of chemicals, industrial process, or occupational exposure cannot be classified as to its carcinogenicity to humans.

10. REFERENCES

Alguire, D.E. 1973. Ethylene oxide gas. Food Technology. pp. 64-67.

Altshuller, A.P. and J.J. Bufalini. 1965. Photochemical aspects of air pollution: A review. Photochem. Photobiol. 4: 97-146.

Anderson, S.R. 1971. Ethylene oxide toxicity: A study of tissue reactions to retained ethylene oxide. J. Lab. Clin. Med. 77: 346-356.

Ando, A. 1968. Mutation induction in rice by radiation combined with chemical protectants and mutagens. International Atomic Energy Agency, Vienna, Tech. Rep. Ser. 86, pp. 7-15. (Cited in NIOSH, 1977).

Anonymous. 1947. Ethylene oxide poisoning. Ind. Hyg. Newsletter. 7: 3-6.

Anonymous. 1977. New process for preserving wood. Adhesives Age. 5: 56.

Anonymous. 1981a. Chemical Profiles. Ethylene Oxide. Chemical Marketing Reporter. June 8, 1981. p. 9.

Anonymous. 1981b. Chemical Profiles. Ethylene Glycol. Chemical Marketing Reporter. June 15, 1981. p. 9.

Anonymous. 1981c. Key Chemicals. Ethylene Oxide. Chem. Eng. News. July 6, 1981. p. 10.

Anonymous. 1982. Aliphatic Organics Forecast. Chemical Marketing Reporter. January 4, 1982. p. 33.

Appelgren, L., G. Eneroth and C. Grant. 1977. Studies on ethylene oxide: Whole body autoradiography and dominant lethal test in mice. Proc. Eur. Soc. Toxicol. 18: 315-317.

Appelgren, L.E., G. Eneroth, C. Grant, L.E. Lanstrom and K. Tenshasen. 1978. Testing of ethylene oxide for mutagenicity using the micronucleus test in mice and rats. Acta. Pharmacol. Toxicol. 43(1): 69-71.

Balazs, T. 1976. Toxicity of ethylene oxide and chloroethanol. Food and Drug Administration Bylines. 7: 150-155.

Barnard, J.A. and R.K.Y. Lee. 1972. Combustion of n-pentane in a shock tube. Combust. Sci. Technol. 6(3): 143-150.

Bartnicki, E.W. and C.E. Castro. 1969. Biodehalogenation. Pathway for transhalogenation and the stereochemistry of epoxide formation from halohydrins. Biochemistry. 8(12): 4677-4680.

BASF Wyandotte Corporation. 1972. Ethylene oxide handling manual. Report No. 21. BASF Wyandotte Corporation, Wyandotte, Michigan.

Bayes, A.L. 1979. Ethylene oxide one-generation reproduction study. (Carnegie-Mellon Institute of Research). Submission to the Environmental Protection Agency under the Toxic Substances Control Act. Citation number 8EHQ-0379-0248. (Later published as Snelling et al., 1982).

Ben-Yehoshua, S. and P. Krinsky. 1968. Gas chromatography of ethylene oxide and its toxic residues. J. Gas Chromatog. 6: 350-351.

Ben-Yehoshua, S., S. Staler, A. Goldner and P. Krinsky. 1971. Toxic residues in Hayani dates after fumigation with ethylene oxide. Pest. Chem. Proc. Intl. Congr. Pest. Chem. 2nd. 4: 593-605.

Bertsch, W., R.C. Chang and A. Zlatkis. 1974. The determination of organic volatiles in air pollution studies. Characterization of profiles. J. Chromatog. Sci. 12: 175-182.

Binder, H. 1974. Ethylene oxide and chlorohydrin in tobacco and its smoke. Fachliche Mitt. Oesterr. Tabakregie 1974. 15: 294-301.

Binder, H. and W. Lindner. 1972. Determination of ethylene oxide in the smoke of treated and untreated cigarettes. Fachliche Mitt. Oesterr. Tabakregie. 13: 215-220.

Bird, M. 1952. Chemical production of mutations in Drosophila: Comparison of techniques. J. Genet. 50: 480-485.

Biro, L., A. Fishes and E. Price. 1974. Ethylene oxide burns, a hospital outbreak involving 19 women. Arch. Dermatol. 110: 924.

Blackford, J.L. 1974. Acrylonitrile. Chemical Economics Handbook. Stanford Research Institute, Menlo Park, California.

Blackford, J.L. 1976a. Propylene oxide. Chemical Economics Handbook. Stanford Research Institute, Menlo Park, California.

Blackford, J.L. 1976b. Ethylene oxide. Chemical Economics Handbook. Stanford Research Institute, Menlo Park, California.

Blackwood, J. and E. Erskine. 1938. Carboxide poisoning. U.S. Navy Med. Bull. 36: 44-45.

Bogan, D.J. and C.W. Hand. 1978. Absolute rate constant, kinetic isotope effect, and mechanism of the reaction of ethylene oxide with oxygen (^3P) atoms. J. Phys. Chem. 82: 2067-2073.

Bridie, A.L., C.J.M. Wolff and M. Winter. 1979. The acute toxicity of some petrochemicals to goldfish. Water Res. 13: 623-626.

Bronsted, J.N., M. Kilpatrick and M. Kilpatrick. 1929. Kinetic studies on ethylene oxides. J. Amer. Chem. Soc. 51: 428-461.

Brown, R.H. and C.J. Purnell. 1979. Collection and analysis of trace organic vapour pollutants in ambient atmospheres: The performance of a Tenax GC adsorbent tube. J. Chromatog. 178: 79-90.

Brown, A.M., C. Bruch, E. Jackson, K. Krell, B. Page and M. Thomas. 1979. Increased mutation frequency due to ethylene oxide adsorbed to plastics. In vitro. 15: 220-221.

Brown, D. 1970. Determination of ethylene oxide and ethylene chlorohydrin in plastic and rubber surgical equipment sterilized with ethylene oxide. J. Assoc. Off. Anal. Chem. 53: 263-267.

Browning, J.B. 1982. Union Carbide Corporation. (Aug. 5) Letter to Office of Toxic Substances, U.S. Environmental Protection Agency.

Bruch, C. 1973. Industrial Sterilization. Phillips, G. and W. Miller, eds. Duke University Press, Durham, North Carolina. pp. 119-123.

Bruch, C. and M. Koesterer. 1961. The microbicidal activity of gaseous propylene oxide and its application to powdered or flaked foods. J. Food Sci. 26: 428.

Carlson, R.M. and R. Caple. 1977. Chemical/biological implications of using chlorine and ozone for disinfection. EPA-600/3-77-066, Environmental Research Laboratory, Duluth, Minnesota.

Carpenter, C., H. Smyth and U. Pozzani. 1949. The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. J. Ind. Hyg. Toxicol. 31: 343-349.

Casteignau, G. and J.L. Halary. 1972. Identification et dosage par chromatographic enphase gazause d'oxetanues disubstitues en 3 et de quelques autres ethers-oxydes. Bull. Soc. Chem. Fr. 420-428.

Castro, C.E. and E.W. Bartnicki. 1968. Biodehalogenation. Epoxidation of halohydrins. Epoxide opening and transhalogenation by a flavobacterium sp. Biochemistry. 7: 3213-3218.

Cawse, J.N., J.P. Henry, M.W. Swartzlander and P.H. Wadia. 1980. Ethylene Oxide. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Grayson, M. and D. Eckroth, eds. John Wiley and Sons, Inc., New York. vol. 9, pp. 432-471.

Chemical Week: 1982 Buyers' Guide Issue. 1981. McGraw-Hill, Inc., New York. p. 417.

Clarke, C., W. Davidson and J. Johnson. 1966. Hemolysis of blood following exposure to an Australian manufactered plastic tubing sterilized by means of ethylene oxide gas. Australia (South) New Zealand J. Surg. 36: 53-55.

Cobis, J. 1977. Correspondence from the Department of Medicine and Surgery (Code 134D), Veterans Administration, Washington, D.C., March 17, 1977. (Cited in Glaser, 1977).

Cogswell, S.A. 1980. Chemical Economics Handbook. Marketing Research Report on Ethylene Oxide. SRI International, Menlo Park, CA. January, 1980. pp. 654.5032S, 654.5032Q, 654.5032U.

Conan, L., B. Foucault, G. Siou, M. Chalgneau and G. LeMoan. 1979. Study of the mutagenic action of ethylene oxide, ethylene glycol, and 2-chloroethanol residues in plastic material sterilized by ethylene oxide. Ann. Falsif. Expert. Chim. 72: 141-151.

Conway, R.A., G.T. Waggy, M.H. Spiegel and R.L. Berglund. 1983. Environmental fate and effects of ethylene oxide. Environ. Sci. Technol. 17: 107-112.

Crump, K.S., H.A. Guess and L.L. Deal. 1977. Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. Biometrics. 33: 437-451.

Crutzen, P.J. and J. Fishman. 1977. Average concentrations of OH in the troposphere and the budgets of CH_4, CO, H_2, and CH_3CCl_3. Geophys. Res. Lett. 4: 321-324.

Cumming, R.B., G.A. Sega, C.Y. Horton and W.H. Olson. 1981. Degree of alkylation of DNA in various tissues of the mouse following inhalation exposure to ethylene oxide. Environ. Mutagen. 3(3): 343.

Cumming, R.B., T.A. Michand, L.R. Lewis and W.H. Olson. 1984. Inhalation mutagenesis in mammals. I. Patterns of unscheduled DNA synthesis induced in germ cells of male mice by exposure to ethylene oxide in air. (In press). (Mutagen. Res.).

Cupitt, L.T. 1980. Fate of toxic and hazardous materials in the air environment. U.S. EPA. EPA-600/3-80-084. NTIS PB 80-221948, 35 pp.

Cupitt, L.T. 1983. Personal communication between Dr. L. Cupitt of ESRL and Dr. D.A. Gray of Syracuse Research Corporation. March 1983.

Curme, G. and F. Johnston (eds.). 1952. Glycols. American Chemical Society Monograph, Series 114. Reinhold, New York.

Cvetanovic, R.J. 1955. Mercury photosensitized decomposition of ethylene oxide. Can. J. Chem. 33: 1684-1695.

Darnall, K.R., A.C. Lloyd, A.M. Winer and J.N. Pitts, Jr. 1976. Reactivity scale for atmospheric hydrocarbons based on reaction with hydroxyl radical. Environ. Sci. Technol. 10: 692-696.

DeBont, J.A.M. and R.A.J.M. Albers. 1976. Microbial metabolism of ethylene. Antonie van Leeuwenhoek. 42(1): 78-80.

Dilling, W.L. 1977. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylene from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11: 405-409.

Dimitriades, B. and S.B. Joshi. 1977. Application of reactivity criteria in oxidant-related emission control in the U.S.A. In: Inter. Conf. on Photochemical Oxidant Pollution and Its Control. Dimitriades, B., ed. Research Triangle Park, North Carolina. pp. 705-711. EPA-600/3-77-011B.

Dobbs, A.J., B.C. Gilbert and R.O.C. Norman. 1971. Electron spin resonance studies. Part XXVII. The geometry of oxygen-substituted alkyl radicals. J. Chem. Soc. A: 124-135.

Dobinson, B., W. Hoffman and B.D. Stack. 1969. The Determination of Epoxide Groups. Pergamon Press, Oxford.

Dolovich, J. and B. Bell. 1978. Allergy to a product(s) of ethylene oxide gas: Demonstration of IgE and IgG antibodies and hapten specificity. J. Allerg. Clin. Immunol. 62: 30-32.

Dow Chemical Co. 1977. Alkylene oxides from Dow. Dow Chemical Co. Form No. 110-551-77R, Midland, Michigan.

Dunkelberg, H. 1979. On the oncogenic activity of ethylene oxide and propylene oxide in mice. Br. J. Cancer. 39(4): 588-589.

Dunkelberg, H. 1981. Kanzerogene Aktivitat von Ethylenoxid und seinen Reaktions-produkten 2-Chloroethanal, 2-Bromoethanol, Ethylenglykol und Diethylenglykol. I. Kanzerogenitat von Ethylenoxid im Vergleich zu 1,2-Propylenoxid bei Subkutaner Applikation an Mausen. Zbl. Bakt. Hyg. I. Abt. Orig. B 174: 383-404.

Dunkelberg, H. 1982. Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. Br. J. Cancer. 46: 924-933.

Eastham, A.M. and G.A. Latremouille. 1952. Ethylene oxide V reaction with halide ions in neutral and acid solution. Can. J. Chem. 30: 169.

Ehrenberg, L. and A. Gustaffson. 1957. On the mutagenic action of ethylene oxide and diepoxybutane in barley. Hereditas. 43: 595-602.

Ehrenberg, L. and T. Hallstrom. 1967. Haematologic studies on persons occupationally exposed to ethylene oxide. In: International Atomic Energy Agency Report SM 92/96, pp. 327-334.

Ehrenberg, L. and S. Hussain. 1981. Genetic toxicity of some important epoxides. Mutat. Res. 86: 1-113.

Ehrenberg, L., A. Gustaffson and U. Lundquist. 1959. The mutagenic effect of ionizing radiation and reactive ethylene derivatives in barley. Hereditas. 45: 351-368. (Cited in NIOSH, 1977).

Ehrenberg, L., K. Hiesche, S. Osterman-Golker and I. Wennberg. 1974. Evaluation of genetic risks of alkylating agents: Tissue doses in the mouse from air contaminated with ethylene oxide. Mutat. Res. 24: 83-103.

Ehrenberg, L., A. Gustaffson and U. Lundqvist. 1956. Chemically induced mutation and sterility in barley. Acta. Chem. Scand. 10: 492-494. (Cited in NIOSH, 1977).

Embree, J. and C. Hine. 1975. Mutagenicity of ethylene oxide. Toxicol. Appl. Pharmacol. 33: 172-173.

Embree, J., J. Lyon and C.H. Hine. 1977. The mutagenic potential of ethylene oxide using the dominant lethal assay in rats. Toxicol. Appl. Pharmacol. 40: 261-267.

EOIC (Ethylene Oxide Industry Council of the Chemical Manufacturer's Association). 1984. Research directed at the development of a sensitive analytical method for the quantification of ethylene oxide and its metabolites in biological samples. Final report. Prepared by Arthur D. Little, Inc., Cambridge, MA.

Faberge, A.C. 1955. Types of chromosome aberrations induced by ethylene oxide in maize. Genetics. 40: 571. (Cited in NIOSH, 1977).

Fahmy, O. and M. Fahmy. 1970. Gene elimination in carcinogenesis: Reinterpretation of the somatic mutation theory. Cancer Res. 30: 195-205.

Fishbein, L. 1976. Potential hazards of fumigant residues. Environ. Health Perspect. 14: 39-45.

Fomenko, V.N. and E.E. Strekalova. 1973. Mutagenic action of some industrial poisons as a function of concentration and exposure time. Toksikol. Nov. Prom. Khim. Veshchestv. 13: 51-57.

Francis, M. 1985. Letter to Herman Gibb, Carcinogen Assessment Group, U.S. Environmental Protection Agency. Subject: Search request on methyl formate, 107-31-3. Environmental Mutagen, Carcinogen, and Teratogen Information Program, Oak Ridge National Laboratory, Oak Ridge, TN.

Freed, V.H., C.T. Chiou and R. Haque. 1977. Chemodynamics: Transport and behavior of chemicals in the environment - A problem in environmental health. Environ. Health Perspec. 20: 55-70.

Fritz, B., K. Lorenz, W. Steinert and R. Zellner. 1982. Laboratory kinetic investigations of the tropospheric oxidation of selected industrial emissions. Comm. Eur. Communities (Rep.) Eur. Phys.-Chem. Behav. Atmos. Pollut. 7624: 192-202.

Gaines, Personal Communication. Union Carbide Corporation. 203-794-2000.

Garry, V.V., J. Hozier, D. Jacobs, R.L. Wade and D.G. Gray. 1979. Ethylene oxide: Evidence of human chromosomal effects. Environ. Muta. 1: 375-382.

Garry, V.F., C.W. Opp, J.K. Wiencke and D. Lakatua. 1982. Ethylene oxide induced sister chromatid exchange in human lymphocytes using a membrane dosimetry system. Pharmacol. 25: 214-221.

Garry, V.F., C.W. Opp and J.K. Wiencke. 1981. Ethylene-oxide induced sister chromatid exchange in human-lymphocytes using a membrane dosimetry system. Environ. Mutagen. 3(3): 340.

Generoso, W.M., K.T. Cain, M. Krishna, C.W. Sheu and R.M. Gryder. 1980. Heritable translocation and dominant-lethal mutation induction with ethylene oxide in mice. Mutat. Res. 73(1): 133-142.

Gilmour, R.H. 1978. Ethylene oxide treatment of cosmetics. Soap Perfum. Cosmet. 51(11): 498-499.

Gomer, R. and W.A. Noyes. 1950. Photochemical studies. XLII. Ethylene oxide. J. Am. Chem. Soc. 72: 101-108.

Goncarlovs, V. 1983. Personal communication between Dr. D.A. Gray of Syracuse Research Corporation and V. Goncarlovs of the Office of Pesticides. March, 1983.

Graedel, T.E. 1978. Chemical Compounds in the Atmosphere. Academic Press, New York. pp. 267, 272, 276.

Greaves-Walker, W. and C. Greeson. 1932. The toxicity of ethylene oxide. J. Hyg. 32: 409-416.

Gritter, R.J. and E.C. Sabatino. 1964. Free-radical chemistry of cyclic ethers. VII. Ultraviolet photolysis of epoxides and propylene sulfide in the liquid phase. J. Org. Chem. 29: 1965-1967.

Gross, J., M. Haas and T. Swift. 1979. Ethylene oxide neurotoxicity: Report of four cases and review of the literature. Neurology. 29: 978-982.

Hackett, P.L., M.G. Brown, R.L. Buschbom et al. 1982. Teratogenic study of ethylene and propylene oxide and n-butyl acetate. Battelle, Pacific Northwest Laboratories. Prepared for NIOSH, contract number 210-80-0013.

Hansch, C. and A.C. Leo. 1979. Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, Inc., New York. p. 176.

Hatch, G.G., P.D. Mamay, C.C. Christensen, R. Lagenbach, C.R. Goodheart and S. Nesnow. 1982. Enhanced viral transformation of primary Syrian hamster lung (V-79) cells exposed to ethylene oxide in sealed treatment chambers. Proc. Am. Assoc. Cancer Res. 23: 74.

Hawley, G.G. (ed.). 1981. The Condensed Chemical Dictionary, 10th ed., Van Nostrand Reinhold Company, New York. p. 435.

Hemminki, K., P. Mutinen, I. Saloniemi, M.L. Niemi and H. Vainis. 1982. Spontaneous abortions in hospital staff engaged in sterilizing instruments with chemical agents. Br. Med. J. 285: 1461-1463.

Hendry, D.G., T. Mill, L. Piszkiewicz, J.A. Howard and H.K. Eigenmann. 1974. A critical review of H-atom transfer in the liquid phase. J. Phys. Chem. Ref. Data. 3: 937-978.

Hine, C. and V. Rowe. 1973. Industrial Hygiene and Toxicology. 2nd ed. vol. 2, Interscience, New York. pp. 1600-1601.

Hine, C., V.K. Rowe, E.R. White, K.I. Darmer and G.T. Youngblood. 1981. Expoxy Compounds. In: Toxicology. Clayton, G.D. and F.E. Clayton, Eds. Volume 2A of Patty's Industrial Hygiene and Toxicology. Third revised edition. John Wiley and Sons, Inc., NY. pp. 2141-2258.

Hirose, T., R. Goldstein and C. Bailey. 1963. Hemolysis of blood due to exposure to different types of plastic tubing and the influence of ethylene oxide sterilization. J. Thorac. Cardiov. Surg. 45: 245-251.

Hirose, C. 1974. The microwave spectra and r_0, r_s, and r_m structures of ethylene oxide. Bull. Chem. Soc. Japan. 47: 1311-1318.

Hogstedt, C., O. Rohlen, B.S. Berndtson, O. Axelson and L. Ehrenberg. 1979a. A cohort study of mortality and cancer incidence in ethylene oxide production workers. Br. J. Ind. Med. 36: 276-280.

Hogstedt, C., N. Malmgrist and B. Wadman. 1979b. Leukemia in workers exposed to ethylene oxide. J. Am. Med. Assoc. 241(11): 1132-1133.

Hogstedt, C., L. Aringer and A. Gustavasson. 1984. Ethylene oxide and cancer: A literature review and follow-up of two epidemiologic studies. Accepted for publication in Arbete och Halse.

Hollingsworth, R., V. Rowe, F. Oyen, D. McCollister and H. Spencer. 1956. Toxicity of ethylene oxide determined on experimental animals. Am. Med. Assoc. Arch. Ind. Health. 13: 217-227.

Holmberg, P.C. 1979. Central-nervous-system defects in children born to mothers exposed to organic solvents. Lancet. 2: 8135: 177-179.

Holmberg, P.C. and M. Nurminen. 1980. Congenital defects of the nervous system and occupational factors during pregnancy. A case-reference study. Am. J. Ind. Med. 1: 167-176.

Holmgren, A., N. Diding and G. Samuelsson. 1969. Ethylene oxide treatment of crude drugs. Part V. Formation of ethylene chlorohydrin. Acta Pharmacol. Sciencia. 6: 33-36.

Howe, R.B. 1983. GLOBAL 83. An experimental program developed for the U.S. Environmental Protection Agency as an unpdate to GLOBAL 82: A computer program to extrapolate quantal animal toxicity data to low doses (May 1982). K.S. Crump and Co., Inc., Ruston, LA. Unpublished.

Hughes, K.J., R.W. Hurn and F.G. Edwards. 1959. Separation and identification of oxygenated hydrocarbons in combustion products from automotive engines. In: Proc. 2nd Int. Symp. on Gas Chromatography, East Lansing, Michigan, p. 171-182.

Hughes, T.W., D.R. Tierney and Z.S. Khan. 1979. Measuring fugitive emissions from petrochemical plants. Chem. Eng. Prog. August 1979, pp. 35-39.

Hussain, S. and L. Ehrenberg. 1975. Prophage inductive efficiency of alkylating agents and radiations. Intl. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 27: 355-362.

IARC (International Agency for Research on Cancer) 1976. Ethylene oxide. In: IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France.

IARC (International Agency for Research on Cancer) 1982. IARC Monographs: Evaluation of the Carcinogenic Risk of Chemicals to Humans. Supplement 4. Lyon, France.

Ishishi, E. and Y. Kobayashi. 1973. Treatment of a waste solution obtained in the manufacture of epoxide by chlorohydrination. Japan Kokai 73, 10,012, 5 pp.

Jacobson, K., E. Hackley and L. Feinsilver. 1956. The toxicity of inhaled ethylene oxide and propylene oxide vapors. Arch. Ind. Health. 13: 237-244.

Jana, M.K. and K. Roy. 1975. Effectiveness and efficiency of methyl methanesulfonate and ethylene oxide for the induction of mutations in mice. Mutagen. Res. 28(2): 211-215. (Cited in NIOSH, 1977).

Jay, W.M., T.R. Swift and D.S. Hull. 1982. Possible relationship of ethylene oxide exposure to cataract formation. Am. J. Ophthalmol. 93(6): 727-732.

Jefferson Chemical Co. Undated a. Ethylene oxide technical brochure. Houston, Texas.

Jefferson Chemical Co. Undated b. Propylene oxide technical brochure. Houston, Texas.

Jensen, E. 1977. Union Carbide Corp. (unpublished). (Cited in Federal Register, 43: 3812, 1978).

Jenssen, D. and C. Ramel. 1980. The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mutat. Res. 75(2): 191-202.

Johnson and Johnson. 1982. Preliminary report of pilot research chromosome study of workers at sites where ethylene oxide gas is utilized as a sterilant. Unpublished report available from Dr. J. Paul Jones, Director of Health Sciences, Johnson and Johnson, New Brunswick, NJ, p. 18.

Jones, A.R. and G. Wells. 1981. The comparative metabolism of 2-bromoethanol and ethylene oxide in the rat. Xenobiotica. 11(11): 763-770.

Jones, L.A. and D.M. Adams. 1981. Mutations in Bacillus subtilis var. niger (Bacillus globigii) spores induced by ethylene oxide. Sporulation Germination, Proc. Int. Spore Conf. VIII. Levinson, H.S., A.L. Sonenshein, and D.J. Tipper, Eds. American Society of Microbiology, Washington, DC.

Jones-Price, C., C.A. Kimmel, T.A. Marks, T.A. LeDoux, J.R. Reel, P.W. Fisher, L. Langhoff-Paschke and M. Marr. Teratologic evaluation of ethylene oxide (CAS No. 75-71-8) in New Zealand White rabbits. Final report (RB80--EO). NIEHS Contract NO1-ES-2127.

Joshi, S.B., M.C. Dodge and J.J. Bufalini. 1982. Reactivities of selected organic compounds and contamination effects. Atmos. Environ. 16: 1301-1310.

Joyner, R. 1964. Chronic toxicity of ethylene oxide. Arch. Environ. Health. 8: 700-710.

Jukes, T.H. 1964. Choline. Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. Interscience, New York. 5: 405.

Kadoi, M. and I. Kataoka. 1968. Purification of a waste liquor from production of an oxirane compound. Japan Patent 74, 25,119, 5 pp.

Kauhanen, K. 1977. Stanford Research Institute (unpublished). (Cited in Federal Register, 43: 3912, 1978).

Klapproth, E.M. 1976. Ethylene glycol. Chemical Economics Handbook. Stanford Research Institute, Menlo Park, California.

Kligerman, A.D., G.L. Erenson, M.E. Phelps and J.L. Wilmer. 1983. Sister-chromatid exchange induction in peripheral blood lymphocytes of rats exposed to ethylene oxide by inhalation. Mutat. Res. 120: 37-44.

Kolmark, H.G. and B.J. Kilbey. 1968. Kinetic studies of mutation induction by epoxides in Neurospora crassa. Mol. Gen. Genet. 101: 89-98.

Koskikallio, J. and E. Whalley. 1959. Effect of pressure on the spontaneous and the base-catalyzed hydrolysis of epoxides. Can. J. Chem. 37: 783-787.

Krell, K., E.D. Jacobson and K. Selby. 1979. Mutagenic effect on L5178Y mouse lymphoma cells by growth in ethylene oxide-sterilized polycarbonate flasks. In Vitro. 15(5): 326-328.

Kurginski, E.R. 1979. Personal Communication to C. Glasgow, U.S. Environmental Protection Agency, October 22. Dow Chemical Co., Midland, Michigan.

LaBorde, J.B. and C.A. Kimmel. 1980. The teratogenicity of ethylene oxide administered intravenously to mice. Toxicol. Appl. Pharmacol. 56(1): 16-22.

LaBorde, J.B., C.A. Kimmel, C. Jones-Price, T.A. Marks, and T.A. Ledoux. 1982. Teratogenic evaluation of ethylene chlorohydrin (ECH) in mice and rabbits. Society of Toxicology Meeting, Boston, MA.

Lamaty, G., R. Maleq, C. Selve, A. Sivade and J. Wylde. 1975. Mechanisms of ring opening of oxirans by acids in aqueous and nonaqueous solvents. J. Chem. Soc. Perkin II: 1119-1124.

Lambert, B. and A. Lindblad. 1980. Sister chromatid exchange and chromosome aberrations in lymphocytes of laboratory personnel. J. Toxicol. Environ. Health. 6(5-6): 1237-1243.

Landels, S.P. 1976. Fumigants and nematicides. Chemical Economics Handbook. Stanford Research Institute, Menlo Park, California.

Lapkin, M. 1965. Epoxides. Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. Interscience, New York. 8: 288-289.

Laurent, C.H., J. Frederic and F. Marechal. 1982. Etude des effects cytogenetiques d'intoxication a l'oxyde d'ethylene. C.R. Soc. Biol. 176: 733-735.

Lee, W.R. Unpublished. Final report on project "Relation of mutation frequency to dose of ethylene oxide in germ cells." Submitted to South Carolina Pesticides Epidemiologic Study Center, Preventive Medicine Division, Department of Family Practice, Medical University of South Carolina. 12 pp.

Lichtenstein, H.J. and G.H. Twigg. 1948. Trans. Faraday Soc. 44: 905. (Cited in Mabey and Mill, 1978).

Lichtenwalter, G.D. and G.H. Riesser. 1964. Chlorohydrins. Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. 5: 304-324.

Liepins, R., F. Mixon, C. Hudak and T.B. Parsons. 1977. Industrial process profiles for environmental use: Chapter 6. The Industrial Organic Chemicals Industry. Washington, D.C.: U.S. Environmental Protection Agency. Avail. NTIS PB281-478. pp. 6-370 to 6-373.

Lindgren, D. and L. Vincent. 1966. In: Advances in Pest Control Research. vol. 5, Metcalf, R., ed. Interscience, New York. 85 pp.

Lindgren, D. and K. Sulovska. 1969. The mutagenic effect of low concentrations of ethylene oxide in air (abstract only). Hereditas. 63: 460. (Cited in NIOSH, 1977).

Lindgren, D., L. Vincent and H. Krohne. 1954. Relative effectiveness of ten fumigants to adults of eight species of stored-product insects. J. Econ. Entomol. 47: 923-926.

Lindgren, D.L., W.B. Sinclair and L.E. Vincent. 1968. Residues in raw and processed foods resulting from post-harvest insecticidal treatment. Residue Reviews. 21: 93-95.

Logan, J.A., M.J. Prather, S.C. Wofsy and M.B. McElroy. 1981. Tropospheric chemistry: A global perspective. J. Geophys. Res. 86: 7210-7254.

Long, F.A. and J.G. Pritchard. 1956. Hydrolysis of substituted ethylene oxides in H_2O^{18} solutions. J. Amer. Chem. Soc. 78: 2663-2667.

Long, F.A., J.G. Pritchard and F.E. Stafford. 1957. Entropies of activation, and mechanism for the acid-catalyzed hydrolysis of ethylene oxide and its derivatives. J. Amer. Chem. Soc. 79: 2362-2364.

Loveless, A. and P. Wheatley. 1966. In: Genetic and Allied Effects of Alkylating Agents. Pennsylvania State University Press, University Park, Pennsylvania. vol. 131.

Lynch, D.W., T.R. Lewis, W.J. Moorman, P.S. Sabharwal and J.R. Burg. 1982a. Chronic inhalation toxicity of ethylene oxide in rats and monkeys. A Preliminary Report. Presented at the 21st Annual Society of Toxicology Meeting, Boston, MA, February 22-26, 1982. National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, 4676 Columbia Parkway, Cincinnati, OH.

Lynch, D., T. Lewis, W. Moorman, et al. 1982b. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. National Institute for Occupational Safety and Health, Washington, D.C. (Unpublished draft.)

Lynch, D.W., T.R. Lewis, W.J. Moorman, P.S. Sabharwal, J.B. Lal and J.R. Burg. 1983. Toxic and mutagenic effects of ethylene oxide and propylene oxide on spermatogenic function in Cynomologus monkeys. Society of Toxicology Meeting. Las Vegas, NV.

Mabey, W. and T. Mill. 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. Data. 7(2): 383-415.

Mackay, D. and P.J. Leinonen. 1975. Evaporation of low-solubility contaminants from water bodies to atmosphere. Environ. Sci. Technol. 9: 1178-1180.

Mackey, J. 1968. Mutagenesis in volgare wheat. Hereditas. 59: 505-517. (Cited in NIOSH, 1977).

Mantel, M. and M.A. Schneiderman. 1975. Estimating "safe" levels, a hazardous undertaking. Cancer Res. 35: 1379-1386.

March, J. 1977. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure. 2nd ed. McGraw-Hill Book Co., New York.

Margosches, E.H. May 13, 1983. Memorandum to the Record U.S. Environmental Protection Agency. EtO and the study of spontaneous abortions published in the British Medical J. 20 November 1982.

Martis, L., R. Kroes, T.D. Darby and E.F. Woods. 1982. Disposition kinetics of ethylene oxide, ethylene glycol and 2-chlorethanol in the dog. J. Toxicol. Environ. Health. 10: 847-856.

Matter, B.E. and I. Jaeger. 1975. The cytogenetic basis of dominant lethal mutations in mice. Studies with TEM, EMS, and 6-mercaptopurine. Mutat. Res. 33: 251-260.

MCA (Manufacturing Chemists Association). 1971. Chemical Safety Data Sheet SD-38, Ethylene Oxide. Manufacturing Chemists Association, Washington, D.C.

McDonald, T., K. Kasten, R. Hervey, S. Gregg, A. Borgmann and T. Hurchison. 1977. Acute ocular toxicity for normal and irritated rabbit eyes and subacute ocular toxicity for ethylene oxide, ethylene chlorohydrin, and ethylene glycol. Bull. Parenter. Drug Assoc. 31: 25-32.

McLaughlin, R. 1946. Chemical burns of the human cornea. Am. J. Ophthal. 29: 1355-1362.

Mehra, P.N. and S.K. Mann. 1975. Cytological effects of chemical mutagens on Pterotheca falconeri. Part 2. Monofunctional alkylating agents. Nucleus (Calcutta). L7(3): 167-182. (Cited in NIOSH, 1977).

Migliore, L., A.M. Rossi and N. Loprieno. 1982. Mutagenic action of structurally related alkene oxides on Schizosaccharomyces pombe: The influence, '*in vitro*', of mouse-liver metabolizing system. Mutat. Res. 102: 425-437.

Mishmash, H.E. and C.E. Meloan. 1972. Indirect spectrophotometric determination of nanomole quantities of oxiranes. Anal. Chem. 44: 835-836.

Morgan, R.W., K.W. Claxton, B.J. Divine, S.D. Kaplan and V.B. Harris. 1981. Mortality among ethylene oxide workers. J. Occup. Med. 23(11): 767-770.

Morris, J.C. 1975. Formation of halogenated organics by chlorination of water supplies. EPA 600/1-75-002, U.S. Environmental Protection Agency, Washington, D.C.

Moutschen, J., M. Moutschen-Dahmen and L. Ehrenberg. 1968. Note on the chromosome breaking activity of ethylene oxide and ethyleneimine. Hereditas. 60: 267-269. (Cited in NIOSH, 1977).

NAS (National Academy of Science). 1976. Vapor-phase organic pollutants. National Academy of Sciences, Washington, DC.

NFPA (National Fire Prevention Association). 1975. Hazardous chemicals data. Fire Protection Guide on Hazardous Materials. Boston, Massachusetts.

NIOSH (National Institute for Occupational Safety and Health). 1977. Special occupational hazard review with control recommendations for the use of ethylene oxide as a sterilant in medical facilities. Glaser, Z., 67 pp., Department of Health, Education, and Welfare Publication No. 77-200, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

NIOSH (National Institute for Occupational Safety and Heath). 1981. NIOSH Current Intelligence Bulletin No. 35. Ethylene Oxide (EtO): Evidence of Carcinogenicity, May 22, 1981.

NTP (National Toxicology Program). 1981. National Toxicology Program, 1980. Department of Health and Human Services. U.S. Public Health Service.

Ong, E. 1948. Chemistry and Uses of Insecticides. Reinhold Publishing Co., New York. p. 150.

Oxirane Corporation. Undated. Propylene Oxide Technical Bulletin, Houston, Texas.

Patty, F.A. (ed.). 1973. Industrial Hygiene and Toxicology. 2nd revised edition, vol. 2. Interscience, New York. p. 1626.

Pellizzari, E.D., J.E. Bunch, R.E. Berkley and J. McRae. 1976. Collection and analysis of trace organic vapor pollutants in ambient atmospheres. The performance of a Tenax GC cartridge sampler for hazardous vapors. Anal. Letters. 9: 45-63.

Pero, R.W., T. Bryngelsson, B. Widegren, B. Hogstedt and H. Welinder. 1982. A reduced capacity for unscheduled DNA synthesis in lymphocytes from individuals exposed to propylene oxide and ethylene oxide. Mutat. Res. 104: 193-200.

Pero, R.W., B. Widegren, B. Hogstedt and F. Mitelman. 1981. In vivo and in vitro ethylene oxide exposure of human lymphocytes assessed by chemical stimulation of unscheduled DNA synthesis. Mutat. Res. 83: 271-289.

Pessayre, D. and A. Trevoux. 1978. Collapse after endovascular exploration: The role of ethylene oxide or endotoxin? LaNouvelle Presse Medicale. 39: 3558.

Pfeiffer, E. and H. Dunkelberg. 1980. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. Toxicol. 18: 115-118.

Pfeilsticker, K., G. Fabricius and G. Timme. 1975. Simultaneous gas-chromatographic determination of ethylene oxide, ethylene chlorohydrin, and ethylene glycol in grain. Z. Lebensm-Unters. Forsch. 158: 21-25.

Phillips, C. and S. Kaye. 1949. The sterilizing action of gaseous ethylene oxide. Part I. Review. Am. J. Hyg. 50: 270-279.

Poirier, V. and D. Papadopoulo. 1982. Chromosomal abberations induced by ethylene oxide in a human amniotic cell line in vitro. Mutat. Res. 104: 255-260.

Poothullil, J., A. Shimizu, R. Day and J. Dolovich. 1975. Anaphylaxis from the product(s) of ethylene oxide gas. Ann. Intern. Med. 82: 58-60.

Pritchard, J.G. and F.A. Long. 1956. Hydrolysis of ethylene oxide derivatives in deuterium oxide-water mixtures. J. Amer. Chem. Soc. 78: 6008-6013.

Pritchard, J.G. and I.A. Siddiqui. 1973. Activation parameters and mechanisms of the acid-catalyzed hydrolysis of epoxides. J. Chem. Soc. Perkin II: 452-457.

Qazi, A.H. and N.H. Ketcham. 1977. A new method for monitoring personal exposure to ethylene oxide in the occupational environment. Am. Ind. Hyg. Assoc. J. 38: 635-647.

Rannug, U., R. Goethe and C.A. Wachtmeister. 1976. The mutagenicity of chloroacetaldehyde, chloroethylene oxide, 2-chloroethanol, and chloroacetic acid, conceivable metabolites of vinyl chloride. Chem. Biol. Interact. 12: 251-263.

Rathburn, R.E. and D.Y. Tai. 1981. Technique for determining the volatilization coefficients of priority pollutants in streams. Water Res. 15: 243-250. (Cited in Conway et al., 1983).

Reyniers, V., M. Sacksteder and L. Ashburn. 1964. Multiple tumors in female germ-free inbred albino mice exposed to bedding treated with ethylene oxide. J. Natl. Cancer Inst. 32: 1045-1057.

Roberts, J., L. Allison, P. Prichett and K. Riddle. 1943. Preliminary studies on soil sterilization with ethylene oxide. J. Bacteriol. 45: 40.

Romano, S.J. and J.A. Renner. 1975. Comparison of analytical methods for residual ethylene oxide analysis. J. Pharmacol. Sci. 64: 1412-1417.

Romano, S.J., J.A. Renner and P.M. Leitner. 1973. Gas chromatographic determination of residual oxide by head space analysis. Anal. Chem. 45: 2327-2330.

Rowell, R.M. and D.I. Gutzmer. 1975. Chemical modification of wood: Reactions of alkylene oxides with southern yellow pine. Wood Sci. 7: 240-246.

Rowell, R.M., D.I. Gutzmer, I.B. Sadio and R.E. Kinney. 1976. Effects of alkylene oxide treatments on dimensional stability of wood. Wood Sci. 9: 51-54.

Rowlands, D.G. 1971. The metabolism of contact insecticides in stored grains. II. 1966-1969. Residue Reviews. 34: 91-161.

Roy, K. and M.K. Jana. 1973. Chemically induced chlorophyll mutation in rice. Indian Agric. 17(4): 301-308. (Cited in NIOSH, 1977).

Royce, A. and W. Moore. 1955. Occupational dermatitis caused by ethylene oxide. Br. J. Ind. Med. 12: 169.

Salinas, E., L. Sasich, D.H. Hall, R.M. Kennedy and H. Morriss. 1981. Acute ethylene oxide intoxication. Drug Intel. Clinical Pharmacy. 15(5): 384-386.

Schnorr, T.M. 1982. The health of health care workers: A proportionate mortality study. A thesis in epidemiology presented to the graduate faculties of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Science.

Schultze, H.C. 1965. Ethylene Oxide. Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. Interscience, New York. 8: 523-558.

Scudamore, K.A. and S.G. Heuser. 1971. Ethylene oxide and its persistent reaction products in wheat flour and other commodities: Residues from fumigation or sterilization, and effects of processing. Pest. Sci. 2(2): 80-91.

Sega, G.A., R.B. Cumming, J.G. Owens, C.Y. Horton and L.R. Lewis. 1981. Alkylation pattern in developing mouse sperm, sperm DNA, and protamine after inhalation of ethylene-oxide. Environ. Mutagen. 3(3): 371.

Seizinger, D.E. and B. Dimitriades. 1972. Oxygenates in exhaust from simple hydrocarbon fuels. J. Air Pollut. Contr. Assoc. 22(1): 47-51.

Sexton, R. and E. Henson. 1949. Dermatological injuries by ethylene oxide. J. Ind. Hyg. Toxicol. 31: 297-300.

Sexton, R. and E. Henson. 1950. Experimental ethylene oxide human skin injuries. Arch. Ind. Hyg. Occup. Health. 2: 549-564.

Shenderova, R.I., G.K. Alsopova, I.B. Shaklinazarov and V.A. Linetskii. 1972. Purification of waste waters from simultaneous production of propylene oxide and styrene oxide. Khim. Prom. (Moscow). 48: 746-747.

Shupack, J.L., S.R. Andersen and S.J. Romano. 1981. Human skin reactions to ethylene oxide. J. Lab. Clin. Med. 98(5): 723-729.

Sickles, J.E., II, R.S. Wright, C.R. Sutcliff, A.L. Blackard and D.P. Dayton. 1980. Smog chamber studies of the reactivity of volatile organic compounds. Proc. Ann. Meet. Air Pollut. Contr. Assoc. Paper 80-501, 16 pp.

Sittig, M. 1962. Organic Chemical Processes. The Noyes Press, Pearl River, New Jersey.

Sittig, M. 1965. Acrylic Acid and Esters. Noyes Development Corporation, Park Ridge, New Jersey.

Skeehan, A. 1959. A portable sterilizer for the application of ethylene oxide gas in ophthalmology. Am. J. Ophthalmol. 47: 86.

Smith, H.H. and T.A. Lotfy. 1954. Comparative effects of certain chemicals on Tradescantia chromosomes as observed at pollen tube mitosis. Am. J. Bot. 41: 589-593. (Cited in NIOSH, 1977).

Smyth, H., J. Seaton and L. Fischer. 1941. Single dose toxicity of some glycols and their derivatives. J. Ind. Hyg. Toxicol. 23: 259-268.

Snellings, W., J. Pringle, et al. 1979. Teratology and reproduction studies with rats exposed to 10, 33, or 100 ppm of ethylene oxide. Abstract, Society of Toxicology, 18th meeting, New Orleans, March 1979.

Snellings, W.M., C.S. Weil and R.R. Maronpot. 1981. Final report, ethylene oxide, two years inhalation study. Submitted to the U.S. Environmental Protection Agency by Bushy Run Research Center, Pittsburg, PA.

Snellings, W.M., J.P. Zelenak and C.S. Weil. 1982a. Effects on reproduction in Fischer 344 rats exposed to ethylene oxide by inhalation for one generation. Toxicol. Appl. Pharmacol. 63: 382-388.

Snellings, W.M., R.R. Maronpot, J.P. Zelenak and C.P. Laffoon. 1982b. Teratology study in Fischer 344 rats exposed to ethylene oxide by inhalation. Toxicol. Appl. Pharmacol. 64: 476-481.

Snellings, W.M., C.S. Weil and R.R. Maronpot. 1984a. A subchronic inhalation study on the toxicologic potential of ethylene oxide in B6C3F1 mice. Toxicol. Appl. Pharmacol. 76: 510-518.

Snellings, W.M., C.S. Weil and R.R. Marenpot. 1984b. A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. Toxicol. Appl. Pharmacol. 75: 105-117.

Spasovski, M., V. Khristeva, K. Pernov, V. Kirkov, T. Dryanovska, Z. Panova, G. Bobev, N. Gincheva and S. Ivanova. 1980. Health state of the workers in the production of ethylene and ethylene oxide. Khig. Zdraveopaz. 23(1): 41-47.

Spencer, E.F., Jr. 1971. Pollution control in the chemical industry, Chapter 14. In: Industrial Pollution Control Handbook, H.F. Lund, ed. McGraw-Hill Book Co., New York.

SRI International (Stanford Research Institute International). 1977. A Study of Industrial Data on Candidate Chemicals for Testing. Contract No. 68-01-4109, U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C.

SRI International (Stanford Research Institute International). 1981a. 1981 Directory of Chemical Producers: United States of America. Menlo Park, California: SRI International, pp. 594-595.

SRI International (Stanford Research Institute International). 1981b. 1981 Directory of Chemical Producers: United States of America. Supplement II. Menlo Park, California: SRI International, p. 51.

Star, E.G. 1980. Mutagenic and cytotoxic effect of ethylene oxide on human cell cultures. Zentralbl. Bakteriol. 170(5-6): 548-556.

Star, E.G., J. Caselitz and T. Loening. 1980. The effect of ethylene oxide and chemical disinfectant residues upon the larynx and tracheal mucosa of rabbits. Zentralbl. Bakteriol. 170(5-6): 539-547.

Steinberg, S. 1977. Analytical methods for determining ethylene oxide in plastics. Saf. Health Plastics, Natl. Tech. Conf. Soc. Plastics Eng., pp. 141-146.

Stijve, T., R. Kalsbach and G. Eyring. 1976. Determination of occurrence of ethylene chlorohydrin residues in foods fumigated with ethylene oxide. Mitt. Geb. Lebensmittelunters. Hyg. 67(4): 403-428.

Strekalova, E. 1971. Mutagenic action of ethylene oxide on mammals. Toksikol. Nov. Prom. Khim. Veshchestv. 12: 72-78.

Sulovska, K., D. Lindgren, G. Eriksson and L. Ehrenberg. 1969. The mutagenic effect of low concentrations of ethylene oxide in air. Hereditas. 62(1/2): 264-266. (Cited in NIOSH, 1977).

Sykes, G. 1964. Disinfection and Sterilization. J.P. Lippincott Co., Philadelphia, Pennsylvania, pp. 202-227.

Systems Application, Inc. 1982. Human exposure to atmospheric concentrations of selected chemicals. Appendix A-14 Ethylene Oxide. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Tan, E.L., R.B. Cumming and A.W. Hsie. 1981. Mutagenicity and cytotoxicity of ethylene oxide in the CHO/HGPRT system. Environ. Mutagen. 3: 683-686.

Tanooka, H. 1979. Application of Bacillus subtilis spores in the detection of gas mutagens: A case of ethylene oxide. Mutat. Res. 64(6): 433-435.

Taylor, D.G. 1977a. NIOSH Manual of Analytical Methods, 2nd ed., vol. 2. U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Taylor, D.G. 1977b. NIOSH Manual of Analytical Methods, 2nd ed., vol. 3. U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Taylor, J. 1977. Dermatologic hazards from ethylene oxide. Curtis. 19: 189-192.

Taylor, G. 1979. Mutagenicity testing of ethylene oxide (unpublished). Communication to division directors, NIOSH, January 8, 1979.

Thiess, A. 1963. Considerations of health damage through the influence of ethylene oxide. Arch. Toxikol. 20: 127-140.

Thiess, A.M., H. Schwegler, I. Fleig and W.G. Stocker. 1981. Mutagenicity study of workers exposed to alkylene oxides (ethylene oxide - propylene oxide) and derivatives. J. Occup. Med. 23(5): 343-347.

Thiess, A.M., R. Frentzel-Beyme, R. Link and W.G. Stocker. 1982. Mortality study on employees exposed to alkylene oxides (ethylene oxide/propylene oxide) and their derivatives. In: Proceedings of the International Symposium on Prevention of Occupational Cancer, Helsinki, Finland. p. 249-259.

TSCA (Toxic Substances Control Act). 1980. TSCA Chemical Assessment Series. Chemical Screening: Initial Evaluations of Substantial Risk Notices, Section 8(e). U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington, D.C.

Tyler, T.R. and J.A. McKelvey. 1980. Dose-dependent disposition of C^{14} labeled ethylene oxide in rats. Carnegie-Mellon Institute of Research, Pittsburgh, PA.

U.S. EPA. 1976. Frequency of Organic Compounds Identified in Water. EPA-600/4-76-, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1980. Investigation of selected potential environmental contaminants: Epoxides. U.S. Environmental Protection Agency, Washington, DC. EPA-560/11-80-005.

U.S. EPA. 1984. Performance evaluation of the Sciex $TAGA^R$ 600 mobile laboratory for ambient air monitoring. Summary report. Advanced Analysis Techniques Branch, Environmental Monitoring Div., Environmental Monitoring Systems Laboratory, RTP, NC.

U.S. EPA. 1984. Porposed guidelines for carcinogen risk assessment. Fed. Reg. 49(227): 46294-46301.

USITC (U.S. International Trade Commission). 1976. Synthetic Organic Chemicals: United States Production and Sales, 1974. USITC Publication 776, p. 203.

USITC (U.S. International Trade Commission). 1977a. Synthetic Organic Chemicals: United States Production and Sales, 1975. USITC Publication 804, p. 198.

USITC (U.S. International Trade Commission). 1977b. Synthetic Organic Chemicals: United States Production and Sales, 1976. USITC Publication 833, p. 303.

USITC (U.S. International Trade Commission). 1978. Synthetic Organic Chemicals: United States Production and Sales, 1977. USITC Publication 920, p. 361.

USITC (U.S. International Trade Commission). 1979. Synthetic Organic Chemicals: United States Production and Sales, 1978. USITC Publication 1001, p. 313.

USITC (U.S. International Trade Commission). 1980. Synthetic Organic Chemicals: United States Production and Sales, 1979. USITC Publication 1099, p. 269.

USITC (U.S. International Trade Commission). 1981. Synthetic Organic Chemicals: United States Production and Sales, 1980. USITC Publication 1183, p. 265.

USTC (U.S. Tariff Commission). 1974. Synthetic Organic Chemicals: United States Production and Sales, 1972. TC Publication 681, p. 206.

USTC (U.S. Tariff Commission). 1975. Synthetic Organic Chemicals: United States Production and Sales, 1973. TC Publication 728, p. 205.

Van Duuren, B., L. Orris and N. Nelson. 1965. Carcinogenicity of epoxides, lactones, and peroxy compounds, II. J. Natl. Cancer Inst. 35: 707-717.

Veith, G.D., K.J. Macek, S.R. Petrocelli and J. Carroll. 1979. Federal Register 15926 (March 15).

Verrett, M.J. 1974. Memorandum from M.J. Verrett, Reproduction Physiology Branch, FDA, to Tibor Balazs, Bureau of Drug, FDA. Investigation of the toxic and teratogenic effects of 2-chloroethanol to the developing chicken embryo.

Verschueren, K. 1977. Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinhold, NY. p. 322.

Virtanen, P.O.I. and T. Kuokkanen. 1973. Effect of pressure on the rates of acid-catalyzed methanolysis of 3-substituted 1,2-epoxypropanes. Suom. Kemistilehti B. 46(10): 267-270.

von Oettingen, W. 1939. Ethylene oxide. In: Supplement to Occupation and Health: Encyclopedia of Hygiene, Pathology, and Social Welfare. Geneva, International Labor Office.

Waite, C., F. Patty and W. Yant. 1930. Acute responses of guinea pigs to vapors of some new commercial organic compounds. IV. Ethylene oxide. Publ. Health Rep. 45: 1832-1843.

Wallace, T.J. and R.J. Gritter. 1963. Free radical chemistry of cyclic ethers. IV. Free radical rearrangement of epoxides. Tetrahedron. 19: 657-665.

Walpole, A. 1958. Carcinogenic action of alkylating agents. Ann. N.Y. Acad. Sci. 68: 750-761.

Watson, W.A.F. 1966. Further evidence of an essential difference between the genetical effects of mono- and bifunctional alkylation agents. Mutat. Res. 3: 455-457.

Weast, R.C. 1972. Handbook of Chemistry and Physics. 53rd ed. The Chemical Rubber Co., Cleveland, Ohio. p. C-287.

Wesley, F., B. Rourke and O. Darbishire. 1965. The formation of persistent toxic chlorohydrins in foodstuffs by fumigation with ethylene oxide and with propylene oxide. J. Food Sci. 30: 1037.

Whelton, R., H. Phaff, E. Mruk and C. Fisher. 1946. Control of microbiological food spoilage by fumigation with epoxides. Food Ind. 18: 23-25.

Wolman, S.R. 1979. Mutational consequences of exposure to ethylene oxide. J. Environ. Pathol. Toxicol. 2: 1289-1303.

Woodward, G. and M. Woodward. 1971. Toxicity of residuals from ethylene oxide gas sterilization. Proc. of the Health Ind. Assoc. Tech. Symp., Washington, DC. pp. 140-161.

Yager, J.L.W. 1982. Sister chromatid exchange as a biological monitor for workplace exposure: A comparative study of exposure-response to ethylene oxide. Berkeley, CA: The University of California. Northern California Occupational Health Center Report No. NCOHC 82-B-2. March 1982. p. 163. Ph.D. Dissertation.

Yager, J.L.W. and D. Benz. 1982. Sister chromatid exchanges induced in rabbit lymphocytes by ethylene oxide after inhalation exposure. Environ. Mutagen. 4: 121-134.

Yager, J.W., C.J. Hines and R.C. Spear. 1983. Exposure to ethylene oxide at work increases sister chromatid exchanges in human peripheral lymphocytes. Science. 219: 1221-1223.

Yakabova, Z.N., N.A. Shamove, F.A. Miftakhove and L.F. Shilova. 1976. Gynecological disorders in workers engaged in ethylene oxide production. Kazan. Med. Zh. 57(6): 558-560.

Young, H. and R. Busbey. 1935. References to the use of ethylene oxide for pest control. U.S. Department of Agriculture, Bureau of Entomology. Plant Quarantine, April 1935.

Zamlauski, M.J. and G.J. Cohen. 1976. The effects of aortic infusion of ethylene oxide renal function in the rat. Toxicol. Appl. Pharmacol. 38(2): 283-295.